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For: Plant GntI sequences and the use thereof for the production of plants having reduced or lacking N-acetyl glucosaminyl transferase I (GnTI) activity	)	

TRANSMISSION OF COPY OF
INTERNATIONAL APPLICATION AND TRANSLATION THEREOF

Assistant Commissioner for Patents
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Sir:

Attached hereto is a copy of (1) Patent Cooperation Treaty Application EP98/08001, from which this application is a continuation, and (2) a translation into English of the specification and claims of PCT/EP98/08001 as well as a translation of an amended claim set which was the subject of a Written Opinion in the PCT and which cover the subject matter now presented in the application in the USA as claims 2-3 and 31-48.

Respectfully submitted,

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VERIFICATION OF A TRANSLATION

I, the below named translator, hereby declare that:

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I am knowledgeable in the English language and in the language in which the below identified international application was filed, and I believe the English translation of the international application No. PCT/EP98/08001 is a true and complete translation of the above identified international application as filed.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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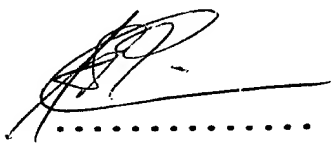

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(translator)

Dr. Antje von Schaewen
EP-81 607/PCT

Plant *GntI* sequences and the use thereof for the production of plants having reduced or lacking N-acetyl glucosaminyl transferase I (GnTI) activity

The present invention relates to plant GnTI sequences, in particular, plant nucleic acid sequences encoding the enzyme N-acetyl glucosaminyl transferase I (GnTI), as well as *GntI* antisense or sense constructs, deduced therefrom, and their translation products, antibodies directed against said translation products as well as the use of the sequence information for the production of transformed microorganisms and of transgenic plants, including those with reduced or lacking N-acetyl glucosaminyl transferase I activity. Such plants with reduced or lacking N-acetyl glucosaminyl transferase I activity are of great importance for the production of glycoproteins of specific constitution with respect to their sugar residues.

Prior art:

In eukaryotes, glycoproteins are cotranslationally assembled in the endoplasmatic reticulum (ER) (i.e. during import into the ER lumen) by the attachment of initially membrane bound glycans (*via* dolichol pyrophosphate) to specific asparagine residues in the growing polypeptide chain (N-glycosylation). In higher organisms, sugar units located at the surface of the folded polypeptide chain are subjected to further trimming and modification reactions (ref. 1) in the Golgi cisternae. Initially, typical basic $Glc_3Man_9GlcNAc_2$ units of the high-mannose type are formed by means of different glycosidases and glycosyl transferases in the ER, which during the passage through the different Golgi cisternae are subsequently converted to so-called complex glycans. The latter are characterized by less mannose units and the presence of

additional sugar residues, such as fucose, galactose and/or xylose in plants and sialic acid (N-acetyl neuraminic acid, NeuNAc) in mammals (ref. 1,2,3). The extent of the modifications can differ between glycoproteins. Single polypetide chains may carry heterogeneous sugar chains. Furthermore, the glycosylation pattern may vary for a specific polypeptide (tissue specific differences), and does not always have to be uniform with respect to a specific glycosylation site, which is referred to as microheterogeneity (ref. 4,5). Up to now, the role of asparagine bound glycans is barely understood, which *i.a.* results from the fact, that said glycans may serve several functions (ref. 6). However, it can be assumed, that e.g. protection of a polypeptide chain from proteolytic degradation can also be achieved by glycans of a more simple oligomannosyl type (ref.7).

Description of problems:

Glycoproteins are highly important in medicine and research. However, large scale isolation of glycoproteins is time-consuming and expensive. The direct use of glycoproteins isolated conventionally often raises problems, since upon administration as a therapeutic, single residues of the glycan components may cause undesired side effects. In this context, the glycan component predominantly contributes to the physico-chemical properties (such as folding, stability and solubility) of the glycoproteins. Furthermore, isolated glycoproteins, as already mentioned above, rarely carry uniform sugar residues, which is referred to as microheterogeneity.

For the production of glycoproteins for medicine and research, yeasts prove to be unsuitable, since they are only able to perform glycosylations of the so-called high-mannose type. While insects and higher plants exhibit complex glycoprotein modifications, these, however, differ from those of animals. Therefore, glycoproteins isolated from plants have a strong antigenic effect in mammals. In most cases, animal organisms

with glycosylation defects are not viable, since terminal glycan residues (e.g. of membraneous glycoproteins) mostly possess biological signal function and are indispensable for cell-cell-recognition during the course of embryogenesis. Mammalian cell lines with defined glycosylation defects already exist, the cultivation of which, however, is labour-intensive and expensive.

For mammals, different glycosylation mutants have been described in detail at the cell culture level (ref. 7,8,9,10). Said mutants are either defective in biosynthesis of mature oligosaccharide chains attached to dolichol pyrophosphate or in glycan processing or show alterations in their terminal sugar residues, respectively. Some of these cell lines exhibit a conditional-lethal phenotype or are defective in intracellular protein transport. The consequences of said defects for the intact organism are difficult to estimate. It has been observed, that a modification in the pattern of complex glycans on the cell surfaces of mammals is accompanied by the formation of tumours and metastases, although a functional relationship could not yet unambiguously be demonstrated (ref. 9). Therefore, in mammals glycosylation mutants are very rare. These defects, summarized under HEMPAS (Hereditary Erythroblastic Multinuclearity with a Positive Acidified Serum lysis test) (ref. 10,11), are based either on a deficiency in mannosidase II and/or low levels of the enzyme N-acetyl glucosaminyl transferase II (GnTII), and have strongly limiting effects on the viability of the mutated organism. *GntI* knock-out mice, in which the gene for GnTI has been destroyed, already die *in utero* of multiple developmental defects (personal communication, H. Schachter, Toronto).

Until recently, no comparable mutants were known for plants. By the use of an antiserum, which specifically recognizes complex modified glycan chains of plant glycoproteins and which predominantly is directed against the highly antigenic

β1→2 linked xylose residues (ref. 12), the applicant was able to isolate several independant mutants from an EMS mutagenized F2 population of the genetic model plant *Arabidopsis thaliana*, which no longer showed complex glycoprotein modification (complex glycan, *cgl* mutants). After at least five back-crosses, each followed by intermittent selfings (to screen for the recessive defects), the glycoproteins were analyzed. These glycoproteins mainly carried glycans of the $Man_5GlcNAc_2$ type, indicating a defect in N-acetyl glucosaminyl transferase I (GnTI) (ref. 8). Indeed, the *Arabidopsis cgl* mutants lacked GnTI activity (ref. 13), which normally catalyzes the first reaction in the synthetic pathway to complex modified sugar chains (ref. 1). However, according to observations so far, the viability of the mutated plants is not affected. In recent publications, plants are suggested as a putative source for the production of pharmaceutically relevant glycoproteins or vaccines (ref. 14,15). There however, it was overlooked, that glycoproteins isolated from plants may cause severe immune reactions in mammals, which up to now obstructed the production of heterologous glycoproteins in cultivated plants.

The applicant could demonstrate by way of example for the *Arabidopsis cgl* mutant, that plants can manage without complex modified glycoproteins to a great extent (ref. 13). Initially, secretory proteins are normally glycosylated in the ER of the mutant. In the Golgi apparatus of the *cgl* mutant, however, the oligomannosyl chains linked to the polypetide backbone *via* asparagine residues (N-glycosylation) then remain at the stage of $Man_5GlcNAc_2$ residues, since N-acetyl glucosaminyl transferase I (GnTI) activity is missing (Fig. 1). By this biosynthesis block, the plant specific complex glycoprotein modification and in particular the attachment of α1→3 fucose and β1→2 xylose residues is prevented, whereby the strong antigenic effect on the mammalian organism is absent. However, Arabidopsis as a herb only has little utilizable biomass. Therefore, for the large scale production of biotechnologically relevant glycoproteins these *cgl* plants are less

suitable. As an alternative, cultivars, especially Solanaceae, such as potato, tobacco, tomato or pepper and furthermore alfalfa, canola, beets, soybean, lettuce, corn, rice and grain, with missing or highly reduced GnTI activity, would be ideal for the production of heterologous glycoproteins in plants. For this purpose, the methods of homology-dependent gene silencing would be applicable (ref. 16, 17).

As Fig. 3 demonstrates, the homology of the first determined plant *GntI* sequence from potato (*Solanum tuberosum* L., St) is extraordinary low in comparison to the corresponding known sequences of animal organisms (only 30-40% identity at the protein level, cf. Fig. 3A). Therefore, by the use of heterologous *GntI* gene sequences an efficient reduction of endogenous complex glycoprotein modification in plants by means of antisense or sense suppression, respectively, (ref. 21), probably cannot be achieved.

Thus, in medicine and research there is still the need for a cost-effective production in suitable organisms of recombinant glycoproteins with a minimum of uniform, i.e. defined sugar residues.

Nature of the present invention:

Since the applicant for the first time has been able to isolate and elucidate plant *GntI* cDNA sequences, it is now possible i.a. to obtain and, in particular, to generate any plant having reduced or missing GnTI activity, and to detect the corresponding mutants, respectively, by means of reverse genetic approaches following transposon (ref. 18) or T-DNA insertion (ref. 19), respectively, so as to produce glycoproteins with low antigenic potential in said mutants.

i) Enzymes

Generally, the present invention comprises different N-acetyl glucosaminyl transferase I enzymes (EC 2.4.1.101) from plants, e.g. potato (*Solanum tuberosum* L.), tobacco (*Nicotiana tabacum* L.) and *Arabidopsis thaliana* (L.). In particular, the present invention relates to enzymes, which exhibit or contain the amino acid sequences given in Fig. 2 and 3B as well as in the accompanying sequence protocol.

Further, the invention comprises enzymes, which are derived from amino acid sequences of the above mentioned enzymes by amino acid substitution, deletion, insertion, modification or by C-terminal and/or N-terminal truncation and/or extension, and which - if showing enzymatic activity - exhibit a specificity comparable to that of the starting enzyme, i.e. N-acetyl glucosaminyl transferase I activity, and optionally a comparable activity.

In the present context, by the term "comparable activity" an activity is understood, which is in the range of up to 100% above or below that of the starting enzyme. Accordingly, also comprised by the invention are derived enzymes or proteins with very low or completely lacking enzymatic activity, which is detectable by means of one or more of the tests mentioned as follows. The enzyme activity is determined by a standard assay, which is performed with microsomal fractions either under radioactive conditions, e.g. using UDP-[6-^{3}H]GlcNAc as a substrate (ref. 13) or non-radioactive conditions (HPLC method; ref 20). Plant GnTI activity can be detected on the subcellular level in Golgi fractions (ref. 21). On account of low yields, however, it is almost impossible to enrich the enzyme from plants.

Alternatively, an enzyme derived according to the present invention, may optionally be defined as an enzyme, for which a DNA sequence encoding the enzyme can be determined or

derived, which hybridizes to a DNA sequence encoding the starting enzyme or to a complementary sequence under stringent conditions, as defined as follows.

For example, an enzyme derived in such a manner represents an isoform, which comprises the amino acids 74 to 446 of the amino acid sequence illustrated in Fig. 2 and in SEQ ID No:1 and 2. This isoform *i.a.* lacks the membrane anchor formed by amino acids 10 to 29. As a result, this enzyme isoform may be located in the plant cytosol.

As examples for C- and/or N-terminally extended proteins, fusion proteins can be mentioned, comprising in addition to an amino acid sequence according to the invention a further protein, which e.g. exhibits a different enzymatic activity or which may be easily detected in another manner, such as by means of fluorescence or phosphorescence or on account of a reactivity with specific antibodies or by binding to suitable affinity matrices.

Furthermore, the invention comprises fragments of said enzymes, which optionally no longer exhibit any enzymatic activity. Generally, these fragments show an antigenic effect in a host immunized with said fragments, and may accordingly be employed as an antigen for the production of monoclonal or polyclonal antibodies by immunization of a host with those fragments.

Moreover, this invention also relates to N-acetyl glucosaminyl transferase I enzymes from other varieties and plant species, which are obtainable on account of hybridization of their genes or one or more regions of their genes:
- to one or more of the DNA sequences and/or DNA fragments of the present invention, as discussed below and/or
- to suitable hybridization probes according to the invention, which may be prepared on the basis of the amino acid sequences mentioned in the sequence protocol considering the degeneration of the genetic code.

Further comprised by the invention in accordance with the above are enzymes or proteins derived from these N-acetyl glucosaminyl transferase I enzymes, including fusion proteins thereof, as well as fragments of all of these enzymes or proteins.

ii) Antibodies

Another aspect of the present invention relates to the use of the amino acid sequences mentioned above and of fragments thereof having antigenic effects, respectively, for the production of monoclonal or polyclonal antibodies or antisera by immunizing hosts with said amino acid sequences or fragments, respectively, as well as of antibodies or antisera, respectively, *per se*, which specifically recognize and bind to the enzymes and/or antigens described above. The general procedure and the corresponding techniques for the generation of polyclonal and monoclonal antibodies are all well-known to the persons skilled in the art.

Exemplarily, by the use of a fragment of the *GntI* cDNA (nucleotides 275 to 1395) represented in Fig. 2 and SEQ ID NO: 1, the recombinant GnTI protein from *Solanum tuberosum* with 10 N-terminal histidine residues (His-tag) was overexpressed in *E. coli*, and, following affinity purification *via* a metal-chelate matrix, was employed as an antigen for the production of polyclonal antisera in rabbits (cf. Examples 5 and 6).

One possible use of the antibodies of the invention resides in the screening of plants for the presence of N-acetyl glucosaminyl transferase I.

Binding of the antibody according to the present invention to plant protein(s) indicates the presence of N-acetyl glucosaminyl transferase I enzyme detectable with said antibody. In

general, this antibody may then be covalently bound to a carrier in a later step, and optionally be employed for the enrichment or purification of the enzyme by means of column chromatography.

On the other hand, a negative binding result using the antibody of the present invention, i.e. lack of binding to the plant proteins, may suggest, that N-acetyl glucosaminyl transferase I enzyme is absent (or highly modified by mutation), and thus, that N-acetyl glucosaminyl transferase I activity of a plant investigated is missing or highly reduced.

Techniques for the realization of the screening assays mentioned above or the enrichment or purification of enzymes by the use of antibody columns or other affinity matrices (cf. Examples 5 and 6) are well-known to those skilled in the art.

iii) DNA sequences

The present invention further comprises DNA sequences encoding the amino acid sequences of the invention, including amino acid sequences derived therefrom according to the above provisions. In particular, the invention relates to the respective gene, which is the basis of the amino acid sequences described in the Figures 2 and 3B and the sequence protocol, and especially, to the cDNA sequences described in Fig. 2 and the sequence protocol, as well as to DNA sequences derived from these genes and DNA sequences.

By the term "derived DNA sequences" are meant sequences, which are obtained by substitution, deletion and/or insertion of one or more and/or smaller groups of nucleotides of the sequences mentioned above and/or by truncation or extension at the 5′ and/or 3′ terminus. Modifications within the DNA sequence may lead to derived DNA sequences, which encode amino acid sequences being identical to the amino acid sequence encoded by the starting DNA sequence, or to such

sequences, in which, compared to the amino acid sequence, which is encoded by the starting DNA sequence, single or a few amino acids are altered, i.e. substituted, deleted and/or inserted, as well as to such sequences, which - optionally in addition - are truncated and/or extended at the C-terminus and/or N-terminus.

Furthermore, the present invention also extends to the complementary sequences of the genes and DNA sequences according to the invention, as well as the RNA transcription products thereof.

Particularly comprised by the present invention are all sequences derived according to the above provisions, which over their entire length or only with one or more partial regions hybridize under stringent conditions to the starting sequences mentioned above or to the sequences complementary thereto or to parts thereof, as well as DNA sequences comprising such sequences.

By the term "hybridization under stringent conditions" in the sense of the present invention is understood a hybridization procedure according to one or more of the methods described below. Hybridizing: up to 20 h in PEG buffer according to Church and Gilbert (0.25 M Na_2HPO_4, 1mM EDTA, 1% (w/v) BSA, 7% (w/v) SDS, pH 7.5 with phosphoric acid; ref. 22) at 42°C or in standard hybridization buffers with formamide at 42°C or without formamide at 68°C (ref. 23). Washing: 3 times at 65°C for 30 min in 3x SSC buffer (ref. 23), 0.1% SDS.

In the sense of the present application, the term "hybridization" always means hybridization under stringent conditions, as mentioned above, even if this is not explicitely indicated in the individual case.

Moreover, the invention relates to fragments of the DNA sequences mentioned above, including the DNA sequences

derived in accordance with the above provisions, to fragments derived from such fragments by nucleic acid substitution, insertion and/or deletion as well as the corresponding fragments with sequences complementary thereto. Such fragments are *i.a.* suitable as sequencing or PCR primers, screening probes and/or for uses as discussed below. For the use as a screening or hybridizing probe, the DNA fragments according to the present invention are frequently employed as radiolabelled fragments. Fragments carrying sequences, which are derived from the starting sequences defined above by substitution, deletion and/or insertion of one or more nucleotides, and the sequences complementary thereto, respectively, are comprised by the invention to that extent, as said fragments hybridize under the above mentioned stringent conditions to the starting sequences, or to the sequences complementary thereto, respectively.

On the basis of the DNA sequences mentioned in the sequence protocol and in Figure 2, DNA fragments according to the invention may for example be obtained starting from plant DNA by means of restriction endonucleases using appropriate restriction sites or by employment of PCR by means of primers appropriately synthesized, or may, as an alternative, also be chemically synthesized. Such techniques are well-known to those skilled in the art.

Moreover, the invention relates to any DNA sequences, which represent a gene or are a part of a gene encoding the enzyme N-acetyl glucosaminyl transferase I and, which in their entirety or in a partial region thereof hybridize under stringent conditions
- to one or more of the DNA sequences of the invention and/or
- to one or more of the DNA fragments of the invention and/or
- to a DNA sequence, which is derived from the amino acid sequences mentioned in the sequence protocol considering the degeneration of the genetic code.

For this purpose, hybridization or screening probes are used as DNA fragments, which generally comprise at least 15 nucleotides, typically between 15 and 30 nucleotides, and, if necessary, substantially more nucleotides. As an example, the primers employed in Example 1 may be used. Alternatively, DNA sequences of appropriate length, derived from the DNA sequences mentioned in the sequence protocol, may be used. As a third possibility, appropriate hybridization probes according to the invention may be developed starting from the amino acid sequences mentioned in the sequence protocol considering the degeneration of the genetic code.

In this respect, a subject-matter of the present invention are also genes encoding N-acetyl glucosaminyl transferase I, which may be detected from other varieties or plant species on account of the hybridization thereof to above mentioned hybridization probes, as well as DNA sequences, DNA fragments and constructs, which are derived therefrom in accordance with the above provisions.

The isolation of the corresponding gene and sequencing thereof following detection by means of the hybridization probes of the invention are well within the skills of a specialist in this field, and are detailed by way of example with respect to N-acetyl glucosaminyl transferase I from *Solanum tuberosum* and to the corresponding enzymes from *Nicotiana tabacum* and *Arabidopsis thaliana* (partial sequence) in the examples.

Finally, another subject matter of the present invention are antisense sequences with respect to any of the above DNA sequences.

iv) Constructs

Also comprised by the invention are constructs, which may optionally comprise besides additional 5′ and/or 3′ sequences, e.g. linkers and/or regulatory DNA sequences or other

modifications, the DNA sequences of the invention, including the DNA sequences derived as detailed above.

An example for this are hybridization or screening probes, which in addition to a DNA sequence of the invention also comprise a detection agent for the verification of hybridization products, which in this case typically is non-radioactive, e.g. fluorescent or phosphorescent molecules, biotin, biotin derivatives, digoxigenin and digoxigenin derivatives. In this respect, however, radioactive or non-radioactive detection agents may be considered, which may be attached to the DNA sequence according to the present invention e.g. by means of end labelling.

A subject-matter of the invention are also antisense and sense constructs with respect to the DNA sequences and fragments according to the present invention, i.e. with respect to

- the DNA sequences mentioned in the sequence protocol and the corresponding genes;
- the DNA sequences derived therefrom in accordance with the above provisions;
- one or more regions of these DNA sequences;
- DNA sequences, especially from other varieties or plant species, which represent a gene or are a part of a gene, encoding the enzyme N-acetyl glucosaminyl transferase I; and which hybridize under stringent conditions
  - -- to one or more of the above DNA sequences and/or
  - -- to one or more of the above DNA fragments and/or
  - -- to a DNA sequence, which is derived from the amino acid sequences mentioned in the sequence protocol considering the degeneration of the genetic code.

Furthermore, the present invention extends to any DNA-transfer systems such as vectors, plasmids, viral and phage genomes or cosmids, which contain the DNA sequences according

to the present invention, e.g. the *GntI* gene, cDNA and DNA regions according to the invention, as mentioned in the sequence protocol, fragments thereof, in particular antisense or sense constructs and/or cDNA sequences derived therefrom according to the above provisions.

Various techniques for the production or synthesis of DNA, DNA fragments, constructs and transfer systems according to the invention, e.g. digestion by means of restriction endonucleases, PCR amplification using suitable primers, optionally followed by cloning and additional chemical or enzymatic modification starting from plant DNA are well-known to those skilled in the art.

One possibility of application of the DNA hybridization probes according to the invention is the detection of N-acetyl glucosaminyl transferase I genes in plants other than those, from which the DNA sequences mentioned in the sequence protocol were obtained, or the detection of potential (other) isoforms of the N-acetyl glucosaminyl transferase I gene in the starting plants *Solanum tuberosum*, *Nicotiana tabacum* and *Arabidopsis thaliana*.

If it is possible to make use of a plant genomic library or cDNA library for the hybridization experiment, a positive hybridization result of such screening of each library may indicate a clone or a few clones, which contain the desired sequence completely or in part, i.e. the N-acetyl glucosaminyl transferase I gene, combined with only a limited amount of other DNA from the genome of the target plant, which appropriately facilitates cloning and sequencing of the target gene. As an alternative, a PCR amplification of the gene or parts thereof may also be carried out starting from plant DNA and suitable constructs, so-called PCR primers, to facilitate cloning and sequencing.

One use of sequencing primers of the invention, which are synthesized starting from suitable regions of the sequences according to the invention, e.g. enables genomic sequencing starting from the entire target plant genomic DNA cleaved by restriction endonucleases, by means of the Church-Gilbert technique, as well as sequencing at the cDNA level following RT-PCR amplification of the total RNA of the target plant (cf. Expl. 1).

An alternative possibility of application of the DNA hybridization probes according to the present invention derived from the DNA sequences mentioned in the sequence protocol, is the use thereof according to the invention for the detection of plants with reduced or lacking N-acetyl glucosaminyl transferase I activity. The hybridization experiment serves to detect the N-acetyl glucosaminyl transferase I (*GntI*) gene by which it may be concluded, e.g. owing to a negative hybridization result under stringent conditions, that the *GntI* gene, and thus, N-acetyl glucosaminyl transferase I activity in a plant investigated is lacking.

Such hybridization techniques for the detection of proteins or genes particularly in plant material by means of DNA probes are also known to the persons skilled in the art. In this context, it is referred to the above statements under item iii) for possible hybridization conditions. Generally, suitable DNA hybridization probes comprise at least 15 nucleotides of a sequence, which for example is derived from the cDNA sequences mentioned in Fig. 2 and the sequence protocol or from the corresponding *GntI* genes.

v) Transformed microorganisms

Furthermore, the invention relates to microorganisms, such as bacteria, bacteriophages, viruses, unicellular eukaryotic organisms, such as fungi, yeasts, protozoa, algae, and human, animal and plant cells, which have been transformed by one or

more of the DNA sequences of the invention or one or more of the constructs of the invention, as illustrated above.

Transformed microorganisms according to the present invention are used e.g. as expression systems for the transforming foreign DNA to obtain the corresponding expression products. For this purpose, typical microorganisms are bacteria, e.g. such as *E. coli*. Furthermore, transformed microorganisms according to the invention, in particular agrobacteria, may be employed e.g. for the transformation of plants by transmission of the transforming foreign DNA.

Methods for the transformation of cells of microorganisms by (foreign) DNA are well-known to those skilled in the art.

For this purpose, e.g. constructs referred to as expression vectors are used, which contain the DNA sequence of the invention under control of a constitutive or inducible promoter, which, if necessary, is additionally tissue specific, so as to enable the expression of the introduced DNA in the target or host cell.

Therefore, a further aspect of the invention is a method for the production of the enzymes and proteins of the invention by using one or more of the transformed microorganisms of the present invention. The method comprises cultivating at least one microorganism transformed by the DNA of the invention, in particular by one of the cDNAs mentioned in the sequence protocol, under the control of an active promoter, as defined above, and isolating the enzyme of the invention from the microorganisms, and, if applicable, also from the culture medium. It is understood, that this method also relates to the production of enzymes and proteins, respectively, which are derived from the enzymes according to the present invention from *Solanum tuberosum*, *Nicotiana tabacum* and *Arabidopsis thaliana*, as defined under i) above.

Methods for the cultivation of transformed microorganisms are well-known to those skilled in the art. For example, the isolation of the expressed enzyme may be employed according to the method described in Example 5 by means of metal-chelate chromatography or, alternatively, by chromatography *via* columns, which contain the antibodies against the enzyme bound to the packing material.

vi) Transgenic plants

Furthermore, the invention comprises transgenic plants, which are transformed by means of a DNA sequence according to the invention or a corresponding construct, respectively. Accordingly, there may be obtained e.g. transgenic plants, in which a GnTI deficiency, for example on account of a missing or defectice *GntI* gene or due to defects in the regulatory regions of this gene, has been removed by complementation using a construct derived from the cDNA sequences mentioned in the sequence protocol, wherein the expression of said construct is under the control of an active constitutive or inducible promoter, which may be additionally tissue specific. In this case, the GnTI enzyme or protein expressed on account of the DNA of the invention contained in the construct and having GnTI activity complements the GnTI activity missing in the starting plant.

Also considered are transgenic plants, in which the GnTI activity already present in the starting plant is increased by additional expression of the *GntI* transgene introduced by means of a construct according to the present invention. Up to now, the extremely low expression of the *GntI* gene *in vivo* accompanied by extremely low enzyme activity, which correspondingly was very difficult to detect, has been a main problem in the investigation of the enzyme N-acetyl glucosaminyl transferase I in plants. The problem of a too low GnTI enzyme activity in plants may be overcome by the coexpression of a DNA according to the present invention.

In this case, it may be preferable for the transformation of plants to employ DNA according to the invention, additionally comprising a sequence region, which following expression enables a facilitated detection and/or enrichment and purification, respectively, of the protein product having GnTI activity. This is for example accomplished by the use of a specific DNA sequence for the expression of a recombinant GnTI enzyme, said sequence carrying a N-terminal or C-terminal sequence extension encoding an affinity marker. If it is additionally intended to provide an amino acid sequence portion between the GnTI enzyme and the affinity marker, which represents a recognition site for a specific protease, cleavage of the N-terminal or C-terminal sequence extension from the GnTI enzyme may be achieved by the subsequent use of this specific protease, and the GnTI enzyme thereby obtained in isolated form.

An example for this is the use of a DNA sequence according to the present invention, which codes for the recombinant GnTI enzyme with a C-terminal sequence extension, encoding the affinity marker AWRHPQFGG (*strep*-tag; ref. 39), and an intervening protease recognition site IEGR. The expression of the DNA according to the present invention provides GnTI enzymes with the C-terminal sequence extension mentioned, by means of which the expressed protein molecules specifically bind to a streptavidin derivatized matrix, and may thus be isolated. Then, by means of the protease factor Xa specifically recognizing the amino acid sequence IEGR, the GnTI portion of the protein molecules may be released. As an alternative, the complete protein may be removed from the streptavidin derivatized matrix by means of biotin or biotin derivatives.

A further example is represented by DNA sequences of the invention, encoding a protein which comprises multiple, e.g. 10, N-terminally added histidine residues (His-tag) in addition to a GnTI enzyme. Due to the N-terminal histidine residues, isola-

tion or purification, respectively, of the proteins expressed may be easily conducted by metal-chelate affinity chromatography (e.g. Ni sepharose) (cf. also Example 5).

Moreover, the invention comprises portions of such transgenic plants, adequately transformed plant cells, transgenic seeds and transgenic reproduction material.

A further important aspect of the invention is the use of the sequence information discussed above for the production of plants having reduced or lacking N-acetyl glucosaminyl transferase I activity.

The possibilities of identifying plants with reduced or lacking N-acetyl glucosaminyl transferase I activity due to a gene defect or a missing gene by means of antibodies of the invention or screening or hybridization probes of the invention have already been described above.

Two additional possibilities reside in the use according to the invention of antisense or sense constructs, respectively, which are derived from the DNA sequence of a plant *GntI* gene, for the production of transgenic plants with reduced or lacking N-acetyl glucosaminyl transferase I activity by means of homology-dependent gene silencing (cf. ref. 16,17). The DNA sequence used as a starting sequence for the generation of the constructs, may be derived from the starting plant to be transformed itself but also from a different plant variety or species. In particular, antisense or sense constructs as discussed under items iii) and iv) above are of use. Generally, the constructs employed comprise at least 50 to 200 and more base pairs.

In particular, the constructs employed for this purpose comprise at least 50 to 200 and more base pairs, with a sequence, which is derived on the basis of

- the cDNA sequences mentioned in the sequence protocol and/or the corresponding *GntI* genes and/or
- the derived DNA sequences discussed above and/or DNA fragments according to the present invention and/or
- the DNA sequences, in particular from other varieties and plant species, which encode N-acetyl glucosaminyl transferase I and which may be identified due to a hybridization under stringent conditions to hybridization or screening probes, as defined under items iii) and iv) above.

Generally, the constructs contain a strong constitutive or inducible promoter, which additionally may be tissue specific, by means of which the antisense or sense DNA sequence regions are controlled.

In the production of transgenic plants by integration of antisense construct(s) into the plant genome or by viral infection of starting plants or plant cells by means of virus containing antisense construct(s) for an extrachomosomal propagation and transcription of the antisense construct or the antisense constructs in infected plant tissue, it is intended to achieve a hybridization of *GntI*-gene transcripts to transcripts of the antisense DNA region at the RNA level, which prevents translation of the *GntI* mRNA. The result is a transgenic plant with strongly decreased contents of N-acetyl glucosaminyl transferase I, and thus, a strongly decreased corresponding enzyme activity.

For the transformation of plants according to the invention with antisense constructs, for example constructs may be employed, which hybridize to one of the complete cDNAs, mentioned in Fig. 2 and in the sequence protocol, or to corresponding regions thereof, generally comprising at least 50 to more than 200 base pairs. Moreover, particularly preferred is the use of fragments, the transcripts of which additionally cause a hybridization to a portion of the 5' untranslated region of the *GntI* mRNA, at which or in the proxi-

mity of which usually the attachment of ribosomes would occur. Examples of such constructs are shown in Fig. 4.

In view of the occurence of an isoform in *Solanum tuberosum*, which probably is located in the cytoplasm due to lack of the membrane anchor (aa 10 to 29) of yet unkown function, it may be desirable to target only the N-acetyl glucosaminyl transferase I enzyme located in the Golgi cisternae, i.e. only that enzyme comprising the membrane anchor. One reason for this desire may be the effort or, in the individual case, also the requirement, to affect as little as possible the cytoplasmatic metabolism of the plant cell, for which the cytoplasmatic N-acetyl glucosaminyl transferase I possibly is of importance. For this purpose, antisense constructs may be used according to the present invention, which themselves or the transcripts of which, respectively, hybridize to a DNA or RNA region of the *GntI* gene or the *GntI* mRNA, comprising a part of the 5' untranslated region and the coding region including the membrane anchor. Generally, the extension of the region of hybridization up to position 266 of the cDNA in Fig. 2 and SEQ ID NO: 1 is considered harmless for the purpose mentioned above.

In the production of transgenic plants by integration of sense constructs into the plant genome or by viral infection of starting plants or plant cells by means of virus containing sense construct(s) for extrachromosomal propagation and expression of the construct or constructs in infected plant tissue, there are assumed hybridization phenomena in tobacco according to the work of Faske et al. (ref. 17), of said constructs to the endogenous *GntI* gene at a posttranscriptional or DNA level, respectively, which finally affect or prevent the translation of the *GntI* gene. Also in this case, the result are transgenic plants having reduced or even lacking N-acetyl glucosaminyl transferase I activity.

Methods for the stable integration of such antisense and sense constructs into the genome of plants, or for the viral infection of plants or plant cells, respectively, for an extrachromosomal propagation and transcription/expression of such constructs in infected plant tissue are well known to those skilled in the art. This includes the direct DNA transfer (e.g. into protoplasts by means of electroporation or by the addition of a high molecular osmotic agent as well as biolistic methods, by which DNA coated particles are shot into the plant tissue), such as the use of natural host/vector systems (e.g. agrobacteria or plant viruses). For viral infection of starting plants or plant cells by viruses containing appropriate constructs for extrachromosomal propagation and transcription/expression of the constructs in infected plant tissue, a variety of specific viruses, such as tobacco mosaic virus (TMV) or potato virus X, is available.

Representative plants, which are suitable for such integration, comprise dicotyledonous as well as monocotyledonous cultivated plants, in particular *Solanaceae* such as potato, tobacco, tomato and pepper. Additionally, banana, alfalfa, canola, beets, soybean, lettuce, corn, rice and grain, would be suitable target plants for the use of homologous antisense constructs. For example, the sequence from *Arabidopsis thaliana* mentioned in the sequence protocol appears to be particularly suitable as a starting sequence for the transformation according to the invention of *Brassicaceae*, such as canola plants, by means of sense or antisense constructs. Further plants of interest are any plants, which express glycoproteins of interest for medicine and research.

Generally, it should be noted, that the transformation according to the invention of plants, which in the corresponding region of the *GntI* gene exhibit a homology of $\geq$ 70% at the nucleotide level to the employed antisense or sense constructs according to the present invention, typically results in

transgenic plants of the invention, which show the desired reduction of N-acetyl glucosaminyl transferase I activity.

Further, another possibility is seen in the targeted destruction (knock-out) of the N-acetyl glucosaminyl transferase I gene *via* gene targeting by means of homologous recombination (ref. 24) in a target plant using a suitable DNA fragment derived from the cDNA sequence of the present invention, similar to the procedure established for yeast systems and mammals.

Further, the present invention comprises transgenic plants, which have been transformed by the antisense or sense constructs mentioned above or the viruses containing the same, respectively, as well as parts of such transgenic plants, correspondingly transformed plant cells, transgenic seeds and transgenic reproduction material.

Methods of the production of transgenic plants, e.g. by means of agrobacteria- or virus-mediated as well as direct DNA transfer are known to those skilled in the art. Concerning representative plants for such a transformation, the above mentioned applies.

The plants of the invention and the plants obtained according to the invention, respectively, with reduced or lacking N-acetyl glucosaminyl transferase I activity, may be used according to the invention for the production of glycoproteins with minimal and uniform, i.e. defined, sugar residues. As discussed above, such glycoproteins are of great importance for medicine and research. As a reasonable source of raw material and food as well as due to their unproblematical disposal *via* composting, plants per se represent ideal bioreactors. According to the present invention, it is now possible to express biotechnologically or pharmaceutically relevant glycoproteins (e.g. therapeutics of low antigenic

potential for mammals) in cultivated plants, in which GnTI activity is highly reduced or completely absent.

Accordingly, the invention also comprises a method for the production of glycoproteins with minimal uniform and defined sugar residues, comprising cultivating a transgenic plant according to the invention, of parts of such plants or of plant cells transformed according to the invention, each expressing the desired glycoprotein, as well as isolating the desired glycoprotein from the cultivated material.

In this context, representative cultivated plants are *Solanaceae*, in particular potato, tobacco, tomato and pepper. Furthermore possible are banana, alfalfa, canola, beets, soybean, lettuce, corn, rice and grain.

The sequence of the enzymatically controlled and plant specific N-glycan modifications, which secretory glycoproteins are subjected to during passage through the Golgi apparatus of higher plants, is schematically shown in Fig. 1. The biosynthesis block due to lacking or insufficient N-acetyl glucosaminyl transferase I (GlcNAc transferase I) activity in a plant leads, instead of complex glycans, to the predominant formation of glycans of the $Man_5GlcNAc_2$ type, i.e. glycoproteins with uniform and well-defined sugar residues, which are of extremely high importance for medicine and research.

For this purpose, the genes encoding the desired glycoproteins may be expressed in their natural producing plants, which have been transformed according to the present invention e.g. by means of antisense or sense constructs to yield transgenic plants with reduced or missing N-acetyl glucosaminyl transferase I activity.

There is also the possibility to use transgenic plants of the invention displaying reduced or lacking N-acetyl glucosaminyl transferase I activity, which additionally have been transformed by the gene encoding the desired glycoprotein. In order to achieve this, constructs may be employed, which contain the gene encoding the desired glycoprotein under the control of a strong constitutive or inducible promoter, which is optionally tissue specific as well, and lead to the integration of the gene into the plant genome. Alternatively, the transformation may also be conducted by viral infection by means of a virus containing the gene for the desired glycoprotein for extrachromosomal propagation and expression of the gene. The glycoprotein may then be expressed in the respective host plant and obtained therefrom.

Naturally, as an alternative, the procedure may be such, that initially a transformation using an expression construct or virus containing the DNA encoding the glycoprotein is performed, and subsequently, another transformation with one or more of the antisense or sense constructs of the invention or with one or more viruses, containing the corresponding DNA, is performed. It is also possible to perform a simultaneous transformation using both constructs or using one virus containing the antisense or sense construct as well as the gene encoding the desired glycoprotein (piggyback version).

Within the scope of the present invention, there is also considered a viral overinfection of the transgenic plants according to the invention, in which integration of an antisense/sense construct and/or the gene encoding the desired glycoprotein into the genome has already occured, by viruses containing the antisense/sense construct and/or the gene encoding the desired glycoprotein, for an additional extrachromosomal propagation and transcription or expression, respectively, of this DNA. As a result, the concentrations of antisense or sense DNA, respectively, or of the expressed glycoprotein may be increased in the transgenic plant cells.

It may prove to be practical for the production according to the invention of glycoproteins with defined glycosylation, to use tissue specific promoters in such cases, where it is intended to obtain the desired glycoproteins specifically only from certain parts of a plant such as tubers or roots. Today, for a large variety of plant tissues, tissue specific promoters are available, which drive expression of foreign genes specifically only in these tissues. By way of example, tuber specific promoters such as patatin class I (ref. 26) and proteinase inhibitor II promoters (ref. 27) may be mentioned. Under certain conditions, both promoters exhibit expression also in leaf tissue, i.e. they can be induced by high metabolite contents (for example sucrose) and in the case of the proteinase inhibitor II promoter also by mechanical lesion or by spraying with abscisic or jasmonic acid, respectively.

The use of tissue specific promoters may also be indicated in cases, where the DNA sequence or the transcription products or translation products thereof according to the invention, respectively, which are employed for the transformation, turn out to be detrimental to certain plant parts, e.g. due to a negative influence on the metabolism of the corresponding plant cells.

As a representative target glycoprotein, human glucocerebrosidase may be used for the therapy of the hereditary Gaucher's disease (ref. 25). In order to obtain human glucocerebrosidase (GC) with uniform and defined sugar residues, e.g. plants of the present invention which are transformed by means of antisense DNA, may be transformed with the gene encoding human glucocerebrosidase. For this purpose, the human glucocerebrosidase cDNA sequence (ref. 38) is modified at the 3' terminus by means of PCR using gene specific primers in a manner, that the recombinant enzyme carries a C-terminal sequence extension encoding an affinity marker (e.g.

AWRHPQFGG, *strep*-tag; ref. 39) and, optionally, also a protease recognition site (e.g. IEGR) between the GnTI enzyme region and the affinity marker. The GC-cDNA sequence thus altered is expressed in *GntI* antisense plants of the present invention by using a strong and optionally tissue specific promoter (e.g. for potato under the control of the tuber specific B33 patatin promoter), so that the enzyme synthesized in these plants exclusively carries well defined N-glycans. The affinity marker is intended to facilitate the enrichment of the recombinant enzyme from the transgenic plants. In this case, the expressed protein molecules (GC-strep molecules) bind to a streptavidin derivatized matrix via the affinity marker sequence and can be released therefrom by means of biotin or biotin derivatives. The removal from the strepatavidin derivatized matrix may also be carried out by means of catalytic amounts of a protease, which exhibits a specificity for the protease recognition site located between the GnTI enzyme region and the affinity marker. In this case, only the GnTI enzyme region is released from the matrix. This could be advantageous especially in that case, if the affinity marker sequence has a detrimental effect on the GnTI activity.

Due to their terminal mannose residues, the $Man_5GlcNAc_2$-glycans of the glucocerebrosidase obtained from the plants of the present invention will be recognized by macrophages as an uptake signal, and can thus directly be employed for the therapy of hereditary Gaucher's disease. Currently, a therapy is only possible upon expensive isolation and deglycosylation of native glucocerebrosidase (ref. 25).

Accordingly, the production of recombinant glycoproteins may be highly facilitated by the use of plant *GntI* sequences compared to conventional methods, e.g. the chemical deglycosylation of purified glycoproteins, which is technically demanding (ref. 25), or a difficult and expensive production in GnTI deficient animal cell lines (ref. 7,10).

Description of the figures:

Fig. 1: Sequence of plant specific N-glycan modifications, which secretory glycoproteins are subject to during passage through the Golgi apparatus of higher plants (ref. 28). The biosythesis block to complex modified glycans is based on a deficiency in GnTI activity (which is either caused by a defective or missing GnTI enzyme or by effective reduction of the *GntI* gene expression) and is indicated by a cross. Meaning of the symbols: (F) fucose residues, (X) xylose residues, (●) GlcNAc residues, (□) mannose residues.

Fig. 2: Full length cDNA sequence of a plant GnTI from potato (*Solanum tuberosum* L.) and amino acid sequence deduced therefrom. By way of example, the complete cDNA of the membrane anchor containing *GntI* isoform from potato leaf tissue (A1) is illustrated. The EcoRI/NotI linkers at the 5′ and 3′ ends of the cDNA are highlighted by bold letters, the binding sites of the degenerate oligonucleotides used for obtaining the RT-PCR probe are underlined. In contrast to already published animal GnTI sequences, the protein sequence derived from the potato cDNA clones contains a potential N-glycosylation site: Asn-X(without Pro)-Ser/Thr, which is indicated by an asterisk. The region of the membrane anchor is highlighted in italics (aa 10 to 29). The start of the isoform (A8), which is potentially located in the cytosol, is indicated by an arrow.

Fig. 3: A, Degree of identity or similarity, respectively, of the amino acid sequence deduced from a complete *GntI* cDNA sequence from potato (A1) in comparison to other GnTI sequences of animal organisms, which have been selected from data bases. Identical amino acid posi-

tions (in %) are printed in bold letters, similar amino acid positions are given in brackets underneath. Meaning of the abbreviations: Hu, human; Ra, rat; Mo, mouse; Ce, *Caenorhabditis elegans* (roundworm); St, *Solanum tuberosum* (potato).
B, Comparison of the derived amino acid sequences of different plant *GntI*-cDNA clones. A_Stb-A1, GnTI from potato leaf; B_Ntb-A9, GnTI from tobacco leaf (A9); C_Atb-Full, GnTI from *Arabidopsis thaliana*. Identical aa are highlighted in black, similar aa in light grey.

Fig. 4: Cloning strategy of the *GntI*-antisense constructs used. Following fill-in of the ends, a NotI linker was introduced into the SalI restriction site of the polylinker region of the plant expression vector pA35 (=pA35N) (ref. 29), and the complete A1-*GntI*-cDNA was inserted into pA35N via NotI. The corresponding antisense construct (=pA35N-A1as) was inserted into binary vector pBin19 (ref. 30) via EcoRI and HindIII. Additionally, following PCR amplification, a 5' fragment of the A1-*GntI*-cDNA comprising 270 bp was cloned into pA35N via XbaI and NotI restriction sites in antisense orientation (=pA35N-A1-short) and also inserted into pBin19. Abbreviations; Numerals in brackets, positions of the restriction sites in the A1-*GntI*-cDNA (in base pairs); pBSK, cloning vector (Stratagene): pGEM3Z, cloning vector (Promega); CaMVp35S, constitutive 35S promoter of cauliflower mosaic virus; OCSpA, polyadenylation signal of octopin synthase; pNOS, promoter of nopaline synthase; NEO, neomycin phosphotransferase (selection marker, confers kanamycin resistance); NOSpA, polyadenylation signal of nopaline synthase; LB/RB, left/right border of the T-DNA of the binary vector; arrow, translation initiation (ATG); A8, start of the GnTI isoform,

which is potentially located in the cytosol (7 aa substitutions in comparison to A1).

Fig. 5: Extent of suppression of complex glycoprotein modification in transgenic potato plants transformed with the long *GntI* antisense construct (cf. Fig. 4). A, Coomassie-stained SDS gel from leaf extracts; B, Western-blot analysis (Ref. 13,33) of parallel samples developed with a complex-glycan antiserum (Ref. 12,13). The lanes contain 30 µg each of total protein: *cgl*(Ara), Arabidopsis *cgl* mutant (Ref. 13); WT(Desi), wild-type potato; the numerals refer to individual transgenic potato plants; the arrows represent molecular weight standards of 66, 45, 36 and 29 kDa, respectively.

Fig. 6: Detection of specificity of the generated GnTI antiserum following cell fractionation (Ref. 40) of tobacco callus material. For Western-blot analysis (Ref. 13,33) 30 µg of protein were applied per lane. The antiserum was used in 1:1000 dilution. Lane 1, homogenate following separation of cellular debris; lane 2, vesicle fraction following column chromatography; lane 3, sucrose gradient fraction I (microsomes); lane 4, sucrose gradient fraction II (plastids); lane 5, antigen used for immunization (recombinant GnTI fusion protein); arrow, molecular weight of about 49 kDa.

Explanation of the abbreviations used in the text:

Aa, amino acid(s); bp, base pair(s); EMS, Ethyl methane sulfonate (mutagenic agent); F2, second filial generation; Fuc, fucose; Glc, glucose; GlcNAc, N-acetyl glucosamine; GnTI, N-acetyl glucosaminyl transferase I (EC 2.4.1.101); *GntI*, gene for GnTI (nuclear encoded); kDa, kilodalton; Man, mannose;

PCR, polymerase chain reaction; PAGE, polyacrylamide gel electrophoresis; ref., reference; RT-PCR, reverse transcription coupled polymerase chain reaction; SDS, sodium dodecyl sulfate; var., variety; Xyl, xylose.

In the following, the invention will be described in more detail by means of examples, which are only intended to illustrate the invention and shall not limit the invention in any manner.

Example 1

Isolation and characterization of plant *GntI* cDNA clones.

Total RNA was isolated from potato and tobacco leaf tissue, and cDNA fragments of about 90 bp were amplified by means of RT-PCR in combination with degenerate primers (procedure analogous to ref. 31), which were derived from conserved amino acid regions of known GnTI sequences frome animal organisms (sense primer 1*, 5'-TG(CT) G(CT)I (AT)(GC)I GCI TGG (AC)A(CT) GA(CT) AA(CT)-3'; antisense primer 3*, 5'-CCA ICC IT(AG) ICC (ACGT)G(CG) (AG)AA (AG)AA (AG)TC-3'; 30 pmol of each primer per 50 µl PCR assay at an annealing temperature of 55°C and 45 cycles). Following gel elution, the ends of the PCR products were repaired (i.e. blunt ended using DNA polymerase I and phosphorylated using T4 polynucleotide kinase) and cloned into the EcoRV restriction site of pBSK (Stratagene). By comparison with known GnTI sequences between the primers (arrows), the identity of the derived amino acid sequences from the potato and tobacco RT-PCR products could be confirmed as being homologous; ⇒Q(R/M)QFVQDP(D/Y)ALYRS⇐ (homologous aa are underlined). Of one clone each, radiolabelled probes were synthesized by means of PCR (standard PCR assay using degenerate primers as above, nucleotide mixture without dCTP, but instead with 50 µCi α-^{32}P-dCTP [>3000 Ci/mMol]), and different cDNA libraries were screened for *GntI* containing clones using the corresponding homologous potato or tobacco probes, respectively (procedure analogous

to ref. 31; the stringent hybridization conditions have already been described in the text above). The cDNA libraries were prepared from mRNA of young and still growing plant parts (sink tissues). Following cDNA synthesis and ligating EcoRI/NotI adaptors (cDNA synthesis kit, Pharmacia) EcoRI compatible lambda arms were ligated, those packaged and used to transfect *E. coli* XL1 Blue cells (Lambda ZAPII cloning and packaging system, Stratagene). Following amplification of the libraries, one full-length *GntI* clone each was isolated from a potato leaf sink library (A1 according to Fig. 2 and SEQ ID NO: 1) and a tobacco leaf sink library (A9 according to SEQ ID NO: 3), as well as two additional clones from a tuber sink library (A6, A8). The deduced GnTI amino-acid sequences contain a potential N-glycosylation site, Asn-X(without Pro)-Ser/Thr, in contrast to those of animals. One of the tuber *GntI* cDNA sequences carries stop codons in all three reading frames in front of the first methionine (A8). The coding region shows high homology to the longer tuber clone (A6) (only 2 aa substitutions), but displays a completely different 5′ non-translated region. Furthermore, the membrane anchor characteristic for the Golgi enzyme is missing, so that this GnTI isoform might be located in the cytosol. Sequence comparisons carried out by means of the gap or pileup option, respectively, and the box option of the gcg software package (J. Devereux, P. Haeberli, O. Smithies (1984) Nucl. Acids Res. 12: 387-395) indicate, that the deduced plant GnTI amino-acid sequences exhibit only 30-40% identity and 57-59% similarity to those of animal organisms (Fig. 3A), while they are highly homologous among each other (75 - 90% identity, Fig. 3B).

The procedure in the case of *Arabidopsis thaliana* was analogous, wherein for the preparation of a specific probe first a partial *GntI* sequence was amplified by RT-PCR using *GntI* sense primer 4A (5′-ATCGGAAAGCTT**GGATCC** CCA GTG GC(AG) GCT GTA GTT GTT ATG GCT TGC-3′; HindIII restriction site underlined, BamHI printed in bold) and antisense primer 3*, as defined

above. First, a 5'-incomplete cDNA clone was isolated from a phage library (Lambda Uni-Zap) using this probe. By means of a vector insert PCR, the missing 5'-terminus was amplified from another library (via an unique SpeI restriction site in the 5' region) and assembled to yield a full-length cDNA sequence. The nucleic acid sequence determined by means of sequencing is listed in SEQ ID NO: 5.

Example 2

Functional complementation of a GnTI defect using *GntI* cDNA upon transient expression in protoplasts of the *Arabidopsis thaliana cgl* mutant.

Approximately 4 weeks subsequent to sowing, protoplasts were isolated from leaves of *cgl* mutants cultivated under sterile conditions (nonstainer plants following 5 backcrosses, ref. 13), transformed with expression constructs of the complete *GntI* cDNA sequences (NotI cDNA fragments, cf. Fig. 4) in sense (pA35N-A1s or pA35N-A9s, respectively) or antisense orientation (pA35N-A1as or pA35N-A9as, respectively), and cultivated for 96 h at room temperature in the dark (50 µg of plasmid DNA each per 1 million protoplasts, PEG method according to ref. 32). Subsequent SDS-PAGE of the protoplast extracts and Western-blot analysis (analogous to ref. 13, 33) indicated functional complementation of the GnTI defect, i.e. complex glycosylation of numerous protein bands upon transient expression of the potato A1 and tobacco A9 sense constructs, but not of the corresponding antisense constructs in protoplasts of the Arabidopsis *cgl* mutant (data not shown).

Example 3

Cloning of the binary expression constructs pBin-35-A1as and pBin-35-A1-short (cf. Fig. 4).

Into the SalI restriction site of the polylinker region (corresponding to the one of pUC18) of plant expression vector pA35 (ref. 29), a NotI linker was introduced subsequently to the fill-in of the ends (=pA35N), and the complete A1-*GntI*-cDNA (nucleotides 9 to 1657, according to the cDNA in Fig. 2) was inserted into pA35N *via* NotI (sense construct pA35N-A1s and antisense construct pA35N-A1as, respectively). The expression cassettes of the sense and antisense constructs, respectively, were isolated via the terminal restriction sites (filled-in NcoI restriction site, partial post digestion with HindIII) as a fragment of about 2410 bp and inserted into the EcoRI (filled-in) and HindIII restriction sites of the binary vector pBin19 (Ref. 30) (=pBin-35-A1s and pBin-35-A1as, respectively). The EcoRI restriction site of the vector is restored by fusion with the equally filled-in NcoI restriction site of the fragment. By means of a standard PCR assay (sense primer: KS sequencing primer (Stratagene) extended for PCR, 5'-GGC CCC CCC TCG AGG TCG ACG GTA TCG-3'; antisense primer: 5'-GGGCC<u>TCTAGA</u>*CTCGAG* AGC (CT)AC TAC TCT TCC TTG CTG CTG GCT AAT CTT G-3', XbaI restriction site underlined, XhoI restriction site in italics), there was additionally amplified a 5'-fragment of the *GntI* cDNA at an annealing temperature of 50°C (nucleotides 9 to 261, according to the cDNA in Fig. 2 and SEQ ID NO: 1). The PCR product was digested with XbaI (within the antisense primer) and NotI (within the 5'-linker of the cDNA), isolated as a fragment of about 260 bp and cloned into pA35N (=pA35N-A1-short). The expression cassette of the short antisense construct was also inserted into pBin19 (=pBin-35-A1-short) as a EcoRI/HindIII fragment (about 1020 bp).

Example 4

Transformation of agrobacteria by means of the binary *GntI* constructs and regeneration of transgenic potato and tobacco plants, respectively, from infected leaf discs.

The binary antisense *GntI* constructs (pBin-35-A1as and pBin-35-A1-short) were transformed into the Agrobacterium strain GV2260 (ref. 34, 35). By way of example, sterile leaf discs of potato plants var. Désirée and of tobacco plants var. Wisconsin 38 were infected with the recombinant agrobacterial lines (50 µl of a fresh overnight culture in 10 ml liquid 2MS medium: 2% sucrose in Murashige & Skoog salt/vitamin standard medium, pH 5.6; small pieces of leaf without midrip; co-cultivation for 2 days in the dark in phytotrons). Subsequent to washing of the infected leaf pieces in 2MS medium with 250 µg/ml claforan, transgenic plants were regenerated from said pieces in tissue culture under kanamycin selection (potato protocol ref. 26; tobacco protocol ref. 36) and analyzed for reduced GnTI activity (exemplary shown in Fig. 5 for transgenic potato plants). As apparent from Fig. 5, antisense suppression of complex glycoprotein modifiaction was successful in transgenic potato plant #439. The determined reduction of complex glycoprotein modification was stable in this transformant over the entire investigation period of several months and has been verified in three tests which were performed in an interval of about 1 month each. For the respective transgenic tobacco plants, analogous results were obtained.

Example 5

Production of recombinant potato GnTI protein (for the production of antibodies).

Recombinant GnTI carrying 10 additional N-terminal histidine residues (His-tag) was produced in *E. coli* by means of the pET system (Novagen) and purified by metal-chelate affinity chromatography. A cDNA fragment comprising nucleotides 275-

1395 of the potato *GntI* cDNA (corresp. to aa 75-446, Fig. 2 and SEQ ID NO: 1 and 2, respectively) was amplified by standard PCR (annealing temperature of 50°C, 30 cycles, ref. 31) (sense primer *GntI*-5'fus: 5'-CATGGATCC CTC GAG AAG CGT CAG GAC CAG GAG TGC CGG C-3'; antisense primer *GntI*-3'stop: 5'-ATCCCGGGATCCG *CTA* CGT ATC TTC AAC TCC AAG TTG-3'; XhoI and BamHI restriction sites, respectively, are underlined, stop codon in italics), and inserted into vector pET16b (Novagen) (=pET-His-A1) *via* the restriction sites of the synthetic primer (5'-XhoI-*GntI*-BamHI-3'). Following propagation and analysis in *E. coli* XL1-Blue (Stratagene) the construct was stored as a glycerol culture. Competent *E. coli* BL21(DE3) pLysS cells (Novagen) were transformed with pET-His-A1 for overexpression. Addtition of IPTG (Isopropyl-1-thio-β-D-galactopyranoside, at 0.5-2 mM) to a BL21 culture in logarithmic growth phase, initially induces the expression of T7 RNA polymerase (from the bacterial chromosome), and thus, also the expression of the recombinant fusion protein under control of the T7 promoter in pET vectors (Novagen). By means of metal-chelate chromatography using TALON matrix (Clontech), recombinant potato GnTI was purified from induced BL21:pET-His-A1 cells under denaturating conditions *via* its His-tag (manufacturer's protocol, Novagen), and the preparation was verified with respect to homogeneity by means of SDS-PAGE.

Example 6
Raising of polyclonal antibodies in rabbits.

Recombinant potato GnTI (from Expl. 5) was used as an antigen. Following the harvest of some milliliters of pre-immune serum, the rabbits were subcutaneously injected with 300-500 µg of affinity-purified protein together with 25 µg of GMDP adjuvant (Gerbu) in intervals of three weeks. Subsequent to three basis injections, the animals were bled from the ear vein 12 to 14 days after the respective successive injection (boost), the serum harvested (ref. 37) and tested for recognition of

recombinant GnTI by Western-blot analyses (dilution 1:200 to 1:2000). The antiserum of the boosts resulting in the lowest background-to-signal ratio were mixed with 0.04% sodium azide, aliquoted and kept at +4°C or for long-term storage at -20°C, respectively. As shown in Fig. 6, Western-blot analyses of tobacco callus cells (BY-2 suspension culture) revealed a specific GnTI signal in enriched microsomal fractions, which indicates, that antibodies raised against the recombinant protein specifially recognize plant GnTI. The detection was carried out with enriched microsomal fractions (ER and Golgi vesicles), since - due to low amounts - it is not possible to detect GnTI protein in crude plant extracts by means of the employed Western-blot method.

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tonoplast intrinsic protein are targeted to vacuoles in leaves of transgenic tobacco. Planta 184: 431-437

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: von Schaewen, Antje Dr. rer. nat.
(B) STREET: Natruperstrasse 169a
(C) CITY: Osnabrueck
(E) COUNTRY: Germany
(F) POSTAL CODE (ZIP): D-49076
(G) TELEPHONE: +49-541-684029

(ii) TITLE OF INVENTION: Plant gntI sequences and the use thereof for the production of plants having reduced or lacking N-acetyl glucosaminyl transferase I (GnTI) activity

(iii) NUMBER OF SEQUENCES: 6

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1669 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Solanum tuberosum
(B) STRAIN: Desiree
(D) DEVELOPMENTAL STAGE: Sink organ
(F) TISSUE TYPE: Mesophyll
(G) CELL TYPE: Leaf cells

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: Lambda ZAP II (Eco RI)
(B) CLONE: gntI-A1(K)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:659..667
(D) OTHER INFORMATION:/function= "Asn codon in this context is a potential glycosylation site"
/product= "N-glycosylation consensus sequence"
/phenotype= "N-glycans modulate protein properties"
/standard_name= "N-glycosylation site"
/label= pot-CHO
/note= "GnTI-coding sequences from animals do not contain this feature"

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(B) LOCATION:53..1393
(C) IDENTIFICATION METHOD: experimental
(D) OTHER INFORMATION:/codon_start= 53
/function= "initiates complex N-glycans on secretory glycoproteins"
/EC_number= 2.4.1.101
/product= "beta-1,2-N-acetylglucosaminyltransferase I"
/evidence= EXPERIMENTAL
/gene= "cgl"
/standard_name= "gntI"
/label= ORF
/note= "first gntI sequence from potato (unpublished)"

(ix) FEATURE:
(A) NAME/KEY: 5'UTR
(B) LOCATION:15..52

(ix) FEATURE:
(A) NAME/KEY: 3'UTR
(B) LOCATION:1394..1655

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:80..139
(D) OTHER INFORMATION:/function= "membrane anchor (amino acids 10-29)"
/product= "hydrophobic amino acid stretch in GnTI"
/standard_name= "membrane anchor of a type II Golgi protein"
/note= "identified by comparison with GnTI sequences from animals"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:1..14
(D) OTHER INFORMATION:/function= "used for cloning the cDNA library in Lambda ZAPII"
/product= "EcoRI/NotI-cDNA adapter"
/number= 1

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:1656..1669
(D) OTHER INFORMATION:/product= "EcoRI/NotI-cDNA adapter"
/number= 2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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Arg Gly Asn Lys Phe Cys Phe Asp Leu Arg Tyr Leu Leu Val Val Ala
              5                  10                  15

GCT CTC GCC TTC ATC TAC ATA CAG ATG CGG CTT TTC GCG ACA CAG TCA        151
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          20                  25                  30

GAA TAT GTA GAC CGC CTT GCT GCT GCA ATT GAA GCA GAA AAT CAT TGT        199
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     35                  40                  45

ACA AGT CAG ACC AGA TTG CTT ATT GAC AAG ATT AGC CAG CAG CAA GGA        247
Thr Ser Gln Thr Arg Leu Leu Ile Asp Lys Ile Ser Gln Gln Gln Gly
 50                  55                  60                  65

AGA GTA GTA GCT CTT GAA GAA CAA ATG AAG CAT CAG GAC CAG GAG TGC        295
Arg Val Val Ala Leu Glu Glu Gln Met Lys His Gln Asp Gln Glu Cys
                70                  75                  80

CGG CAA TTA AGG GCT CTT GTT CAG GAT CTT GAA AGT AAG GGC ATA AAA        343
Arg Gln Leu Arg Ala Leu Val Gln Asp Leu Glu Ser Lys Gly Ile Lys
            85                  90                  95

AAG TTA ATC GGA GAT GTG CAG ATG CCA GTG GCA GCT GTA GTT GTT ATG        391
Lys Leu Ile Gly Asp Val Gln Met Pro Val Ala Ala Val Val Val Met
        100                 105                 110

GCT TGC AGT CGT ACT GAC TAC CTG GAG AGG ACT ATT AAA TCC ATC TTA        439
Ala Cys Ser Arg Thr Asp Tyr Leu Glu Arg Thr Ile Lys Ser Ile Leu
    115                 120                 125

AAA TAC CAA ACA TCT GTT GCA TCA AAA TAT CCT CTT TTC ATA TCC CAG        487
Lys Tyr Gln Thr Ser Val Ala Ser Lys Tyr Pro Leu Phe Ile Ser Gln
130                 135                 140                 145

GAT GGA TCA AAT CCT GAT GTA AGA AAG CTT GCT TTG AGC TAT GGT CAG        535
Asp Gly Ser Asn Pro Asp Val Arg Lys Leu Ala Leu Ser Tyr Gly Gln
                150                 155                 160

CTG ACG TAT ATG CAG CAC TTG GAT TAT GAA CCT GTG CAT ACT GAA AGA        583
Leu Thr Tyr Met Gln His Leu Asp Tyr Glu Pro Val His Thr Glu Arg
            165                 170                 175

CCA GGG GAA CTG GTT GCA TAC TAC AAG ATT GCA CGT CAT TAC AAG TGG        631
Pro Gly Glu Leu Val Ala Tyr Tyr Lys Ile Ala Arg His Tyr Lys Trp
        180                 185                 190

GCA TTG GAT CAG CTG TTT CAC AAG CAT AAT TTT AGC CGT GTT ATC ATA        679
Ala Leu Asp Gln Leu Phe His Lys His Asn Phe Ser Arg Val Ile Ile
    195                 200                 205

CTA GAA GAT GAT ATG GAA ATT GCT GCT GAT TTT TTT GAC TAT TTT GAG        727
Leu Glu Asp Asp Met Glu Ile Ala Ala Asp Phe Phe Asp Tyr Phe Glu
210                 215                 220                 225

GCT GGA GCT ACT CTT CTT GAC AGA GAC AAG TCG ATT ATG GCT ATT TCT        775
Ala Gly Ala Thr Leu Leu Asp Arg Asp Lys Ser Ile Met Ala Ile Ser
                230                 235                 240

TCT TGG AAT GAC AAT GGA CAA AGG CAG TTC GTC CAA GAT CCT GAT GCT        823
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CTT TAC CGC TCA GAC TTT TTT CCT GGT CTT GGA TGG ATG CTT TCA AAA          871
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TCA ACT TGG TCC GAA CTA TCT CCA AAG TGG CCA AAG GCT TAC TGG GAT          919
Ser Thr Trp Ser Glu Leu Ser Pro Lys Trp Pro Lys Ala Tyr Trp Asp
    275                 280                 285

GAC TGG CTA AGG CTG AAA GAA AAT CAC AGA GGT CGA CAA TTT ATT CGC          967
Asp Trp Leu Arg Leu Lys Glu Asn His Arg Gly Arg Gln Phe Ile Arg
290                 295                 300                 305

CCA GAA GTT TGC AGA ACG TAC AAT TTT GGT GAG CAT GGT TCT AGT TTG         1015
Pro Glu Val Cys Arg Thr Tyr Asn Phe Gly Glu His Gly Ser Ser Leu
                310                 315                 320

GGG CAG TTT TTT AAG CAG TAT CTT GAG CCA ATT AAG CTA AAT GAT GTC         1063
Gly Gln Phe Phe Lys Gln Tyr Leu Glu Pro Ile Lys Leu Asn Asp Val
            325                 330                 335

CAG GTT GAT TGG AAG TCA ATG GAC CTA AGT TAC CTT TTG GAG GAC AAC         1111
Gln Val Asp Trp Lys Ser Met Asp Leu Ser Tyr Leu Leu Glu Asp Asn
        340                 345                 350

TAT GTG AAA CAC TTT GGC GAC TTG GTT AAA AAG GCT AAG CCC ATC CAC         1159
Tyr Val Lys His Phe Gly Asp Leu Val Lys Lys Ala Lys Pro Ile His
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370                 375                 380                 385

ATT CAG TAC AGA GAC CAA CTA GAC TTT GAA GAT ATC GCT CGA CAG TTT         1255
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                390                 395                 400

GGC ATT TTT GAA GAA TGG AAG GAT GGT GTA CCA CGG GCA GCA TAT AAA         1303
Gly Ile Phe Glu Glu Trp Lys Asp Gly Val Pro Arg Ala Ala Tyr Lys
            405                 410                 415

GGG ATA GTA GTT TTC CGG TTT CAA ACA TCT AGA CGT GTG TTC CTT GTT         1351
Gly Ile Val Val Phe Arg Phe Gln Thr Ser Arg Arg Val Phe Leu Val
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Ser Pro Asp Ser Leu Arg Gln Leu Gly Val Glu Asp Thr  *
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(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 447 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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Ser Glu Tyr Val Asp Arg Leu Ala Ala Ala Ile Glu Ala Glu Asn His
        35                  40                  45

Cys Thr Ser Gln Thr Arg Leu Leu Ile Asp Lys Ile Ser Gln Gln Gln
    50                  55                  60

Gly Arg Val Val Ala Leu Glu Glu Gln Met Lys His Gln Asp Gln Glu
65                  70                  75                  80

Cys Arg Gln Leu Arg Ala Leu Val Gln Asp Leu Glu Ser Lys Gly Ile
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Lys Lys Leu Ile Gly Asp Val Gln Met Pro Val Ala Ala Val Val Val
            100                 105                 110

Met Ala Cys Ser Arg Thr Asp Tyr Leu Glu Arg Thr Ile Lys Ser Ile
        115                 120                 125

Leu Lys Tyr Gln Thr Ser Val Ala Ser Lys Tyr Pro Leu Phe Ile Ser
    130                 135                 140

Gln Asp Gly Ser Asn Pro Asp Val Arg Lys Leu Ala Leu Ser Tyr Gly
145                 150                 155                 160

Gln Leu Thr Tyr Met Gln His Leu Asp Tyr Glu Pro Val His Thr Glu
                165                 170                 175

Arg Pro Gly Glu Leu Val Ala Tyr Tyr Lys Ile Ala Arg His Tyr Lys
            180                 185                 190

Trp Ala Leu Asp Gln Leu Phe His Lys His Asn Phe Ser Arg Val Ile
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Ala Leu Tyr Arg Ser Asp Phe Phe Pro Gly Leu Gly Trp Met Leu Ser
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Val Gln Val Asp Trp Lys Ser Met Asp Leu Ser Tyr Leu Leu Glu Asp
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Phe Gly Ile Phe Glu Glu Trp Lys Asp Gly Val Pro Arg Ala Ala Tyr
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(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1737 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Nicotiana tabacum
(B) STRAIN: Samsun NN
(D) DEVELOPMENTAL STAGE: Sink organ
(F) TISSUE TYPE: Mesophyll
(G) CELL TYPE: Leaf cells

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: Lambda ZAP II (Eco RI)
(B) CLONE: gntI-A9(T)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:733..741
(D) OTHER INFORMATION:/function= "Asn codon in this context is a potential glycosylation site"
/product= "N-glycosylation consensus sequence"
/phenotype= "N-glycans modulate protein properties"
/standard_name= "N-glycosylation site"
/label= pot-CHO
/note= "GnTI sequences from animals do not contain this feature"

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:127..1467
(C) IDENTIFICATION METHOD: experimental
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"beta-1,2-N-acetylglucosaminyltransferase I"
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/label= ORF
/note= "first gntI sequence from tobacco (unpublished)"

(ix) FEATURE:
(A) NAME/KEY: 5'UTR
(B) LOCATION:15..126

(ix) FEATURE:
(A) NAME/KEY: 3'UTR
(B) LOCATION:1468..1723

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:154..213
(D) OTHER INFORMATION:/function= "membrane anchor (amino acids 10-29)"
/product= "hydrophobic amino acid stretch in GnTI"
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(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:1..14
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/product= "EcoRI/NotI-cDNA adapter"
/number= 1

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:1724..1737
(D) OTHER INFORMATION:/product= "EcoRI/NotI-cDNA adapter"
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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

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                 450                 455                 460

ATC TTG GCT GCT GTC GCC TTC ATC TAC ACA CAG ATG CGG CTT TTT GCG       216
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         480                 485                 490

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Asn His Cys Thr Ser Gln Thr Arg Leu Leu Ile Asp Gln Ile Ser Leu
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510                 515                 520                 525

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Gln Glu Cys Arg Gln Leu Arg Ala Leu Val Gln Asp Leu Glu Ser Lys
                 530                 535                 540

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Gly Ile Lys Lys Leu Ile Gly Asn Val Gln Met Pro Val Ala Ala Val
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Val Val Met Ala Cys Asn Arg Ala Asp Tyr Leu Glu Lys Thr Ile Lys
         560                 565                 570

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Ser Ile Leu Lys Tyr Gln Ile Ser Val Ala Ser Lys Tyr Pro Leu Phe
     575                 580                 585

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Ile Ser Gln Asp Gly Ser His Pro Asp Val Arg Lys Leu Ala Leu Ser
590                 595                 600                 605

TAT GAT CAG CTG ACG TAT ATG CAG CAC TTG GAT TTT GAA CCT GTG CAT       648
Tyr Asp Gln Leu Thr Tyr Met Gln His Leu Asp Phe Glu Pro Val His
                 610                 615                 620

ACT GAA AGA CCA GGG GAG CTG ATT GCA TAC TAC AAA ATT GCA CGT CAT       696
Thr Glu Arg Pro Gly Glu Leu Ile Ala Tyr Tyr Lys Ile Ala Arg His
             625                 630                 635

TAC AAG TGG GCA TTG GAT CAG CTG TTT TAC AAG CAT AAT TTT AGC CGT       744
Tyr Lys Trp Ala Leu Asp Gln Leu Phe Tyr Lys His Asn Phe Ser Arg
         640                 645                 650

GTT ATC ATA CTA GAA GAT GAT ATG GAA ATT GCC CCT GAT TTT TTT GAC       792
Val Ile Ile Leu Glu Asp Asp Met Glu Ile Ala Pro Asp Phe Phe Asp
     655                 660                 665

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670                 675                 680                 685

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Ala Ile Ser Ser Trp Asn Asp Asn Gly Gln Met Gln Phe Val Gln Asp
                690                 695                 700

CCT TAT GCT CTT TAC CGC TCA GAT TTT TTT CCC GGT CTT GGA TGG ATG      936
Pro Tyr Ala Leu Tyr Arg Ser Asp Phe Phe Pro Gly Leu Gly Trp Met
            705                 710                 715

CTT TCA AAA TCT ACT TGG GAC GAA TTA TCT CCA AAG TGG CCA AAG GCT      984
Leu Ser Lys Ser Thr Trp Asp Glu Leu Ser Pro Lys Trp Pro Lys Ala
        720                 725                 730

TAC TGG GAC GAC TGG CTA AGA CTC AAA GAG AAT CAC AGA GGT CGA CAA     1032
Tyr Trp Asp Asp Trp Leu Arg Leu Lys Glu Asn His Arg Gly Arg Gln
    735                 740                 745

TTT ATT CGC CCA GAA GTT TGC AGA ACA TAT AAT TTT GGT GAG CAT GGT     1080
Phe Ile Arg Pro Glu Val Cys Arg Thr Tyr Asn Phe Gly Glu His Gly
750                 755                 760                 765

TCT AGT TTG GGG CAG TTT TTC AAG CAG TAT CTT GAG CCA ATT AAA CTA     1128
Ser Ser Leu Gly Gln Phe Phe Lys Gln Tyr Leu Glu Pro Ile Lys Leu
                770                 775                 780

AAT GAT GTC CAG GTT GAT TGG AAG TCA ATG GAC CTT AGT TAC CTT TTG     1176
Asn Asp Val Gln Val Asp Trp Lys Ser Met Asp Leu Ser Tyr Leu Leu
            785                 790                 795

GAG GAC AAT TAC GTG AAA CAC TTT GGT GAC TTG GTT AAA AAG GCT AAG     1224
Glu Asp Asn Tyr Val Lys His Phe Gly Asp Leu Val Lys Lys Ala Lys
        800                 805                 810

CCC ATC CAT GGA GCT GAT GCT GTC TTG AAA GCA TTT AAC ATA GAT GGT     1272
Pro Ile His Gly Ala Asp Ala Val Leu Lys Ala Phe Asn Ile Asp Gly
    815                 820                 825

GAT GTG CGT ATT CAG TAC AGA GAT CAA CTA GAC TTT GAA AAT ATC GCA     1320
Asp Val Arg Ile Gln Tyr Arg Asp Gln Leu Asp Phe Glu Asn Ile Ala
830                 835                 840                 845

CGG CAA TTT GGC ATT TTT GAA GAA TGG AAG GAT GGT GTA CCA CGT GCA     1368
Arg Gln Phe Gly Ile Phe Glu Glu Trp Lys Asp Gly Val Pro Arg Ala
                850                 855                 860

GCA TAT AAA GGA ATA GTA GTT TTC CGG TAC CAA ACG TCC AGA CGT GTA     1416
Ala Tyr Lys Gly Ile Val Val Phe Arg Tyr Gln Thr Ser Arg Arg Val
            865                 870                 875

TTC CTT GTT GGC CAT GAT TCG CTT CAA CAA CTC GGA ATT GAA GAT ACT     1464
Phe Leu Val Gly His Asp Ser Leu Gln Gln Leu Gly Ile Glu Asp Thr
        880                 885                 890

TAA CAAAGATATG ATTGCAGGAG CCCGGGCAAA ATTTTTGACT TATTGGGTAG          1517
*

GATGCATCGA GCTGACACTA AACCATGATT TTACCAGTTA CATACAACGT TTTAATGTTA   1577

TACGGAGGAG CTCACTGTTC TAGTGTTGAA GGGATATCGG CTTCTTAGTA TTGGATGAAT   1637
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CATCAACACA ACCTATTATT TTAAGTGTTC AGAACATAAA GAGGAAATGT AGCCCTGTAA 1697

AGACTATACA TGGGACCATC ATAATCGCGG CCGCGAATTC 1737

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 447 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Met Arg Gly Asn Lys Phe Cys Cys Asp Phe Arg Tyr Leu Leu Ile Leu
1 5 10 15

Ala Ala Val Ala Phe Ile Tyr Thr Gln Met Arg Leu Phe Ala Thr Gln
20 25 30

Ser Glu Tyr Ala Asp Arg Leu Ala Ala Ala Ile Glu Ala Glu Asn His
35 40 45

Cys Thr Ser Gln Thr Arg Leu Leu Ile Asp Gln Ile Ser Leu Gln Gln
50 55 60

Gly Arg Ile Val Ala Leu Glu Glu Gln Met Lys Arg Gln Asp Gln Glu
65 70 75 80

Cys Arg Gln Leu Arg Ala Leu Val Gln Asp Leu Glu Ser Lys Gly Ile
85 90 95

Lys Lys Leu Ile Gly Asn Val Gln Met Pro Val Ala Ala Val Val Val
100 105 110

Met Ala Cys Asn Arg Ala Asp Tyr Leu Glu Lys Thr Ile Lys Ser Ile
115 120 125

Leu Lys Tyr Gln Ile Ser Val Ala Ser Lys Tyr Pro Leu Phe Ile Ser
130 135 140

Gln Asp Gly Ser His Pro Asp Val Arg Lys Leu Ala Leu Ser Tyr Asp
145 150 155 160

Gln Leu Thr Tyr Met Gln His Leu Asp Phe Glu Pro Val His Thr Glu
165 170 175

Arg Pro Gly Glu Leu Ile Ala Tyr Tyr Lys Ile Ala Arg His Tyr Lys
180 185 190

Trp Ala Leu Asp Gln Leu Phe Tyr Lys His Asn Phe Ser Arg Val Ile
195 200 205

Ile Leu Glu Asp Asp Met Glu Ile Ala Pro Asp Phe Phe Asp Phe Phe
210 215 220

Glu Ala Gly Ala Thr Leu Leu Asp Arg Asp Lys Ser Ile Met Ala Ile
225 230 235 240

Ser Ser Trp Asn Asp Asn Gly Gln Met Gln Phe Val Gln Asp Pro Tyr
245 250 255

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Ala Leu Tyr Arg Ser Asp Phe Phe Pro Gly Leu Gly Trp Met Leu Ser
            260                 265                 270

Lys Ser Thr Trp Asp Glu Leu Ser Pro Lys Trp Pro Lys Ala Tyr Trp
        275                 280                 285

Asp Asp Trp Leu Arg Leu Lys Glu Asn His Arg Gly Arg Gln Phe Ile
    290                 295                 300

Arg Pro Glu Val Cys Arg Thr Tyr Asn Phe Gly Glu His Gly Ser Ser
305                 310                 315                 320

Leu Gly Gln Phe Phe Lys Gln Tyr Leu Glu Pro Ile Lys Leu Asn Asp
                325                 330                 335

Val Gln Val Asp Trp Lys Ser Met Asp Leu Ser Tyr Leu Leu Glu Asp
            340                 345                 350

Asn Tyr Val Lys His Phe Gly Asp Leu Val Lys Lys Ala Lys Pro Ile
        355                 360                 365

His Gly Ala Asp Ala Val Leu Lys Ala Phe Asn Ile Asp Gly Asp Val
    370                 375                 380

Arg Ile Gln Tyr Arg Asp Gln Leu Asp Phe Glu Asn Ile Ala Arg Gln
385                 390                 395                 400

Phe Gly Ile Phe Glu Glu Trp Lys Asp Gly Val Pro Arg Ala Ala Tyr
                405                 410                 415

Lys Gly Ile Val Val Phe Arg Tyr Gln Thr Ser Arg Arg Val Phe Leu
            420                 425                 430

Val Gly His Asp Ser Leu Gln Gln Leu Gly Ile Glu Asp Thr  *
        435                 440                 445
```

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1854 base pairs
(B) TYPE: Nucleotide
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Arabidopsis thaliana
(B) STRAIN: Columbia
(D) DEVELOPMENTAL STAGE: Mature plants
(F) TISSUE TYPE: All tissues

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: Lambda Uni-ZAP (EcoRI/XhoI) and Lambda ACT (XhoI)
(B) CLONE: pBSK-Ara-GntI-full #8

(ix) FEATURE:

(A) NAME/KEY: misc_feature
(B) LOCATION:1185..1193
(D) OTHER INFORMATION:/function= "Asn Codon is a potential glycosylation site"
/product= "Consensus sequence for N-glycosylation"
/phenotype= "N glycans modulate protein characteristics"
/standard_name= "N glycosylation site"
/label= pot-CHO
/note= "absent in animal GnTI sequences"

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:135..1469
(C) IDENTIFICATION METHOD: experimental
(D) OTHER INFORMATION:/codon_start= 135
/function= "initiates complex N glycans on secretory glycoproteins"
/EC_number= 2.4.1.101
/product=
"beta-1,2-N-acetyl glucosaminyl transferase I"
/evidence= EXPERIMENTAL
/gene= "cgl"
/standard_name= "gntI"
/label= ORF
/note= "first gntI sequence from Arabidopsis (unpublished)"

(ix) FEATURE:
(A) NAME/KEY: 5'UTR
(B) LOCATION:19..134

(ix) FEATURE:
(A) NAME/KEY: 3'UTR
(B) LOCATION:1470..1848

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:157..215
(D) OTHER INFORMATION:/function= "membrane anchor (amino acids 8-27)"
/product= "hydrophobic amino-acid region in GnTI"
/standard_name= "membrane anchor of a Type II Golgi protein"
/note= "identified by comparison with animal GnTI sequenzes "

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:1..18
(D) OTHER INFORMATION:/function= "for preparation of a cDNA library in Lambda ACT"
/product= "XhoI-cDNA-Adaptor"
/number= 1

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:1849..1854
(D) OTHER INFORMATION:/product= "XhoI-cDNA-Adaptor"
/number= 2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

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CTCGAGGCCA CGAAGGCCAC CGTTTTTGTT ATAACGAACG ACACCGTTTC AAACAACTTC      60

CTTATTAGCT AGCTCCCTCC CGGCGGCAAA CACCAGAAGA TCCACCGCTT TTGATCTGGT     120

TGTTTGTCGT CGAT ATG GCG AGG ATC TCG TGT GAC TTG AGA TTT CTT CTC        170
                Met Ala Arg Ile Ser Cys Asp Leu Arg Phe Leu Leu
                  1               5                  10

ATC CCG GCA GCT TTC ATG TTC ATC TAC ATC CAG ATG AGG CTT TTC CAG        218
Ile Pro Ala Ala Phe Met Phe Ile Tyr Ile Gln Met Arg Leu Phe Gln
          15                  20                  25

ACG CAA TCA CAG TAT GCA GAT CGC CTC AGT TCC GCT ATC GAA TCT GAG        266
Thr Gln Ser Gln Tyr Ala Asp Arg Leu Ser Ser Ala Ile Glu Ser Glu
      30                  35                  40

AAC CAT TGC ACT AGT CAA ATG CGA GGC CTC ATA GAT GAA GTT AGC ATC        314
Asn His Cys Thr Ser Gln Met Arg Gly Leu Ile Asp Glu Val Ser Ile
  45                  50                  55                  60

AAA CAG TCG CGG ATT GTT GCC CTC GAA GAT ATG AAG AAC CGC CAG GAC        362
Lys Gln Ser Arg Ile Val Ala Leu Glu Asp Met Lys Asn Arg Gln Asp
                  65                  70                  75

GAA GAA CTT GTG CAG CTT AAG GAT CTA ATC CAG ACG TTT GAA AAA AAA        410
Glu Glu Leu Val Gln Leu Lys Asp Leu Ile Gln Thr Phe Glu Lys Lys
              80                  85                  90

GGA ATA GCA AAA CTC ACT CAA GGT GGA CAG ATG CCT GTG GCT GCT GTA        458
Gly Ile Ala Lys Leu Thr Gln Gly Gly Gln Met Pro Val Ala Ala Val
          95                  100                 105

GTG GTT ATG GCC TGC AGT CGT GCA GAC TAT CTT GAA AGG ACT GTT AAA        506
Val Val Met Ala Cys Ser Arg Ala Asp Tyr Leu Glu Arg Thr Val Lys
      110                 115                 120

TCA GTT TTA ACA TAT CAA ACT CCC GTT GCT TCA AAA TAT CCT CTA TTT        554
Ser Val Leu Thr Tyr Gln Thr Pro Val Ala Ser Lys Tyr Pro Leu Phe
  125                 130                 135                 140

ATA TCT CAG GAT GGA TCT GAT CAA GCT GTC AAG AGC AAG TCA TTG AGC        602
Ile Ser Gln Asp Gly Ser Asp Gln Ala Val Lys Ser Lys Ser Leu Ser
                  145                 150                 155

TAT AAT CAA TTA ACA TAT ATG CAG CAC TTG GAT TTT GAA CCA GTG GTC        650
Tyr Asn Gln Leu Thr Tyr Met Gln His Leu Asp Phe Glu Pro Val Val
              160                 165                 170

ACT GAA AGG CCT GGT GAA CTG ACT GCG TAC TAC AAG ATT GCA CGT CAC        698
Thr Glu Arg Pro Gly Glu Leu Thr Ala Tyr Tyr Lys Ile Ala Arg His
          175                 180                 185

TAC AAG TGG GCA CTG GAC CAG TTG TTT TAC AAA CAC AAA TTT AGT CGA        746
Tyr Lys Trp Ala Leu Asp Gln Leu Phe Tyr Lys His Lys Phe Ser Arg
      190                 195                 200

GTG ATT ATA CTA GAA GAC GAT ATG GAA ATT GCT CCA GAC TTC TTT GAT        794
Val Ile Ile Leu Glu Asp Asp Met Glu Ile Ala Pro Asp Phe Phe Asp
  205                 210                 215                 220
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TAC TTT GAG GCT GCA GCT AGT CTC ATG GAT AGG GAT AAA ACC ATT ATG          842
Tyr Phe Glu Ala Ala Ala Ser Leu Met Asp Arg Asp Lys Thr Ile Met
                225                 230                 235

GCT GCT TCA TCA TGG AAT GAT AAT GGA CAG AAG CAG TTT GTG CAT GAT          890
Ala Ala Ser Ser Trp Asn Asp Asn Gly Gln Lys Gln Phe Val His Asp
            240                 245                 250

CCC TAT GCG CTA TAC CGA TCA GAT TTT TTT CCT GGC CTT GGG TGG ATG          938
Pro Tyr Ala Leu Tyr Arg Ser Asp Phe Phe Pro Gly Leu Gly Trp Met
        255                 260                 265

CTC AAG AGA TCG ACT TGG GAT GAG TTA TCA CCA AAG TGG CCA AAG GCT          986
Leu Lys Arg Ser Thr Trp Asp Glu Leu Ser Pro Lys Trp Pro Lys Ala
    270                 275                 280

TAC TGG GAT GAT TGG CTG AGA CTA AAG GAA AAC CAT AAA GGC CGC CAA         1034
Tyr Trp Asp Asp Trp Leu Arg Leu Lys Glu Asn His Lys Gly Arg Gln
285                 290                 295                 300

TTC ATT GCA CCG GAA GTC TGT AGA ACA TAC AAT TTT GGT GAA CAT GGG         1082
Phe Ile Ala Pro Glu Val Cys Arg Thr Tyr Asn Phe Gly Glu His Gly
                305                 310                 315

TCT AGT TTG GGA CAG TTT TTC AGT CAG TAT CTG GAA CCT ATA AAG CTA         1130
Ser Ser Leu Gly Gln Phe Phe Ser Gln Tyr Leu Glu Pro Ile Lys Leu
            320                 325                 330

AAC GAT GTG ACG GTT GAC TGG AAA GCA AAG GAC CTG GGA TAC CTG ACA         1178
Asn Asp Val Thr Val Asp Trp Lys Ala Lys Asp Leu Gly Tyr Leu Thr
        335                 340                 345

GAG GGA AAC TAT ACC AAG TAC TTT TCT GGC TTA GTG AGA CAA GCA CGA         1226
Glu Gly Asn Tyr Thr Lys Tyr Phe Ser Gly Leu Val Arg Gln Ala Arg
    350                 355                 360

CCA ATT CAA GGT TCT GAC CTT GTC TTA AAG GCT CAA AAC ATA AAG GAT         1274
Pro Ile Gln Gly Ser Asp Leu Val Leu Lys Ala Gln Asn Ile Lys Asp
365                 370                 375                 380

GAT GAT CGT ATC CGG TAT AAA GAC CAA GTA GAG TTT GAA CGC ATT GCA         1322
Asp Asp Arg Ile Arg Tyr Lys Asp Gln Val Glu Phe Glu Arg Ile Ala
                385                 390                 395

GGG GAA TTT GGT ATA TTT GAA GAA TGG AAG GAT GGT GTG CCA CGA ACA         1370
Gly Glu Phe Gly Ile Phe Glu Glu Trp Lys Asp Gly Val Pro Arg Thr
            400                 405                 410

GCA TAT AAA GGA GTA GTG GTG TTT CGA ATC CAG ACA ACA AGA CGT GTA         1418
Ala Tyr Lys Gly Val Val Val Phe Arg Ile Gln Thr Thr Arg Arg Val
        415                 420                 425

TTC CTG GTT GGG CCA GAT TCT GTA ATG CAG CTT GGA ATT CGA AAT TCC         1466
Phe Leu Val Gly Pro Asp Ser Val Met Gln Leu Gly Ile Arg Asn Ser
    430                 435                 440

TGA TGCAAAACAT ATGAAAGGAA AAGAAGATTT TGGACCGCAT GCAGCCTCCT              1519
 *
445

TCTAGCAGCT GTTAGGTTGT ATTGTTATTT ATGGATGAGT TTGTAGAGCG GCGGGGTTAA       1579

CTTTAACAGC AAGGAAGCTC TGGTGACCAG GCTGATTGGC TTAGAAGTTA TGGGAACCCC       1639
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TTGAAAGGGT CAGGGTTAAA TATATTTCAG TTGTTTTATT AGTGATTATC TTGTGGGTAA 1699

CTTATACGAA TGCAAATCAT TCTATGCAGT TTTTCTTCGT CCCACTTGTT TTGGCTTCTC 1759

TATTGCTAGT GTACATATCT CTTCAAACAT GTACTAAATA ATGCGTGTTG CTTCAAAGAA 1819

GTAACTTTTA TTAAAAAAAA AAAAAAAAAC TCGAG 1854

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 445 amino acids
(B) TYPE: Amino acid
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Met Ala Arg Ile Ser Cys Asp Leu Arg Phe Leu Leu Ile Pro Ala Ala
1 5 10 15

Phe Met Phe Ile Tyr Ile Gln Met Arg Leu Phe Gln Thr Gln Ser Gln
20 25 30

Tyr Ala Asp Arg Leu Ser Ser Ala Ile Glu Ser Glu Asn His Cys Thr
35 40 45

Ser Gln Met Arg Gly Leu Ile Asp Glu Val Ser Ile Lys Gln Ser Arg
50 55 60

Ile Val Ala Leu Glu Asp Met Lys Asn Arg Gln Asp Glu Glu Leu Val
65 70 75 80

Gln Leu Lys Asp Leu Ile Gln Thr Phe Glu Lys Lys Gly Ile Ala Lys
85 90 95

Leu Thr Gln Gly Gly Gln Met Pro Val Ala Ala Val Val Val Met Ala
100 105 110

Cys Ser Arg Ala Asp Tyr Leu Glu Arg Thr Val Lys Ser Val Leu Thr
115 120 125

Tyr Gln Thr Pro Val Ala Ser Lys Tyr Pro Leu Phe Ile Ser Gln Asp
130 135 140

Gly Ser Asp Gln Ala Val Lys Ser Lys Ser Leu Ser Tyr Asn Gln Leu
145 150 155 160

Thr Tyr Met Gln His Leu Asp Phe Glu Pro Val Val Thr Glu Arg Pro
165 170 175

Gly Glu Leu Thr Ala Tyr Tyr Lys Ile Ala Arg His Tyr Lys Trp Ala
180 185 190

Leu Asp Gln Leu Phe Tyr Lys His Lys Phe Ser Arg Val Ile Ile Leu
195 200 205

Glu Asp Asp Met Glu Ile Ala Pro Asp Phe Phe Asp Tyr Phe Glu Ala
210 215 220

Ala Ala Ser Leu Met Asp Arg Asp Lys Thr Ile Met Ala Ala Ser Ser

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225                 230                 235                 240

Trp Asn Asp Asn Gly Gln Lys Gln Phe Val His Asp Pro Tyr Ala Leu
                245                 250                 255

Tyr Arg Ser Asp Phe Phe Pro Gly Leu Gly Trp Met Leu Lys Arg Ser
            260                 265                 270

Thr Trp Asp Glu Leu Ser Pro Lys Trp Pro Lys Ala Tyr Trp Asp Asp
        275                 280                 285

Trp Leu Arg Leu Lys Glu Asn His Lys Gly Arg Gln Phe Ile Ala Pro
    290                 295                 300

Glu Val Cys Arg Thr Tyr Asn Phe Gly Glu His Gly Ser Ser Leu Gly
305                 310                 315                 320

Gln Phe Phe Ser Gln Tyr Leu Glu Pro Ile Lys Leu Asn Asp Val Thr
                325                 330                 335

Val Asp Trp Lys Ala Lys Asp Leu Gly Tyr Leu Thr Glu Gly Asn Tyr
            340                 345                 350

Thr Lys Tyr Phe Ser Gly Leu Val Arg Gln Ala Arg Pro Ile Gln Gly
        355                 360                 365

Ser Asp Leu Val Leu Lys Ala Gln Asn Ile Lys Asp Asp Asp Arg Ile
    370                 375                 380

Arg Tyr Lys Asp Gln Val Glu Phe Glu Arg Ile Ala Gly Glu Phe Gly
385                 390                 395                 400

Ile Phe Glu Glu Trp Lys Asp Gly Val Pro Arg Thr Ala Tyr Lys Gly
                405                 410                 415

Val Val Val Phe Arg Ile Gln Thr Thr Arg Arg Val Phe Leu Val Gly
            420                 425                 430

Pro Asp Ser Val Met Gln Leu Gly Ile Arg Asn Ser  *
        435                 440                 445
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C L A I M S

1. Method for the production of glycoproteins displaying minimal, uniform and defined sugar residues, comprising cultivating a transgenic plant, parts of transgenic plants or transformed plant cells, and isolating the desired glycoprotein from the material cultivated, characterized in that the transgenic plant, parts of transgenic plants or transformed plant cells, respectively, is/are transformed with an antisense construct or a sense construct, comprising an antisense DNA or a sense DNA with respect to the DNA sequence for a gene or a cDNA for plant N-acetyl glucosaminyl transferase I or a part thereof, for elimination or reduction of the activity of said N-acetyl glucosaminyl transferase, wherein the antisense or sense construct optionally contains additional regulatory sequences for the transcription of the respective antisense or sense DNA.

2. Method according to claim 1, characterized in that for transformation an antisense or sense construct with respect to one of the cDNAs encoding N-acetyl glucosaminyl transferase I from *Solanum tuberosum*, *Nicotiana tabacum* or *Arabidopsis thaliana* is used.

3. Method according to claim 2, characterized in that for transformation an antisense or sense construct with respect to one of the DNA sequences given in SEQ ID NO: 1, 3 or 5 is used.

4. Method according to any of the claims 1 to 3, characterized in that the transgenic plant used is

additionally transformed with the gene encoding the desired glycoprotein.

5. DNA, characterized in that it encodes N-acetyl glucosaminyl transferase I from *Solanum tuberosum*.

6. DNA according to claim 5, characterized in that it comprises the nucleotide sequence given in SEQ ID NO: 1 or a part thereof.

7. DNA, characterized in that it encodes N-acetyl glucosaminyl transferase I from *Nicotiana tabacum*.

8. DNA according to claim 7, characterized in that it comprises the nucleotide sequence given in SEQ ID NO: 3 or a part thereof.

9. DNA encoding N-acetyl glucosaminyl transferase I from *Arabidopsis thaliana*, characterized in that said DNA encodes the amino-acid sequence given in SEQ ID NO: 6 or the nucleotide sequence given in SEQ ID NO: 5 or a part thereof.

10. DNA, characterized in that it comprises the nucleotide sequence complementary to the DNA according to claim 6, 8 or 9.

11. DNA, characterized in that it may be obtained by substitution, deletion and/or insertion of one or more nucleotides and/or truncation at the 5′ and/or 3′ end of one of the DNAs according to any of the claims 5 to 10, with the proviso, that said DNA hybridizes at least in a partial region with the starting DNA or its complementary sequence or parts thereof under stringent conditions.

12. DNA, characterized in that it represents a gene or is part of a gene, which encodes the enzyme N-acetyl glucosaminyl transferase I, and which in its entirety or in a partial region thereof hybridizes under stringent conditions
- to one of the DNA sequences or fragments according to any of the claims 5 to 11 and/or
- to a DNA sequence, which has been derived from the amino acid sequences given in SEQ ID NO: 1, 3 and/or 5, considering the degeneration of the genetic code.

13. DNA construct,
characterized in that it comprises one or more of the DNAs according to any of the claims 5 to 14.

14. DNA construct according to claim 13,
characterized in that it comprises an antisense or sense DNA with respect to the DNA sequence according to any of the claims 5 to 12 and optionally regulatory sequences for the transcription of the antisense or sense DNA, respectively.

15. Vector, plasmid, cosmid, virus or phage genome,
characterized in that it contains at least a DNA and/or construct according to any of the claims 5 to 14.

16. N-acetyl glucosaminyl transferase I from *Solanum tuberosum*.

17. N-acetyl glucosaminyl transferase I from *Nicotiana tabacum*.

18. N-acetyl glucosaminyl transferase I from *Arabidopsis thaliana*, characterized in that the enzyme comprises the amino acid sequence set forth in SEQ ID NO: 6.

19. N-acetyl glucosaminyl transferase I, characterized in that the enzyme comprises the amino acid sequence set forth in SEQ ID NO: 2.

20. N-acetyl glucosaminyl transferase I, characterized in that the enzyme comprises amino acids 74 to 446 of the amino acid sequence set forth in SEQ ID NO: 2.

21. N-acetyl glucosaminyl transferase I, characterized in that the enzyme comprises the amino acid sequence set forth in SEQ ID NO: 4.

22. N-acetyl glucosaminyl transferase I, available due to hybridization of its gene or one or more of the portions of its gene to one or more of the DNAs and/or DNA fragments according to any of the claims 5 to 12.

23. Enzymes or proteins derived from the enzymes according to any of the claims 16 to 22 by substitution, deletion, insertion and/or modification of individual amino acids and/or smaller groups of amino acids and/or by N- and/or C-terminal truncation and/or extension.

24. Protein or peptide, comprising one or more portions of the amino acid sequence(s) of one or more of the enzymes defined in any of the claims 16 to 23.

25. Protein or peptide, encoded by one of the DNAs according to any of the claims 5 to 12.

26. Antigen, characterized in that it comprises:
- the amino acid sequence given in SEQ ID NO: 2, SEQ ID NO: 4 or SEQ ID NO: 6, or
- amino acids 74 to 446 of the amino acid sequence given in Fig. 2, or
- an amino acid sequence derived from the amino acid sequences given in SEQ ID NO: 2, 4 or 6 by substitution,

deletion, insertion and/or modification of individual amino acids and/or smaller groups of amino acids, or
- one or more parts of said sequences,
with the proviso, that upon immunization of a host with the antigen, said antigen may raise an immunological reaction, including the production of antibodies directed against the antigen.

27. Monoclonal or polyclonal antibody, characterized in that it specifically recognizes and binds one or more of the enzymes or antigens according to any of the claims 16 to 26.

28. Microorganism,
characterized in that it is transformed by at least one of the nucleotide sequences selected from the DNAs, constructs, vectors, plasmids, cosmids, virus or phage genomes according to one or more of the claims 5 to 15.

29. Transgenic plant, transgenic seed, transgenic reproduction material, parts of transgenic plants or transformed plant cell, obtainable by integration of one or more DNA sequence(s) or construct(s) according to any of the claims 5 to 13 under the control of a promoter effective in plants, into the genome of a plant, or *via* infection by means of a virus containing one or more DNA sequence(s) or construct(s) according to any of the claims 5 to 13, for an extrachromosomal propagation and expression of the DNA sequence(s) or construct(s) in the plant tissue infected.

30. Transgenic plant, transgenic seed, transgenic reproduction material, parts of transgenic plants or transformed plant cell with missing or reduced N-acetyl glucosaminyl transferase I activity, obtainable by integration of one or more antisense or sense construct(s) according to claim 14 under the control of a promoter

effective in plants, into the genome of a plant, or by viral infection by means of a virus ccntaining one or more antisense or sense construct(s) according to claim 14, for an extrachromosomal propagation and transcription of the antisense construct(s) in the plant tissue infected.

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SUMMARY OF THE INVENTION

This invention relates to plant *GntI* sequences, in particular to plant nucleic acid sequences encoding the enzyme N-acetyl glucosaminyl transferase I (GnTI), DNA sequences derived therefrom, including *GntI* antisense and sense constructs, and the translation products thereof, antibodies directed against said translation products, as well as the use of the sequence information for the production of transformed microorganisms and transgenic plants, including those having reduced or missing N-acetyl glucosaminyl transferase I activity. Such plants displaying reduced or lacking N-acetyl glucosaminyl transferase I activity are of great importance for the production of glycoproteins of specific constitution with respect to their sugar residues.

Figure 1

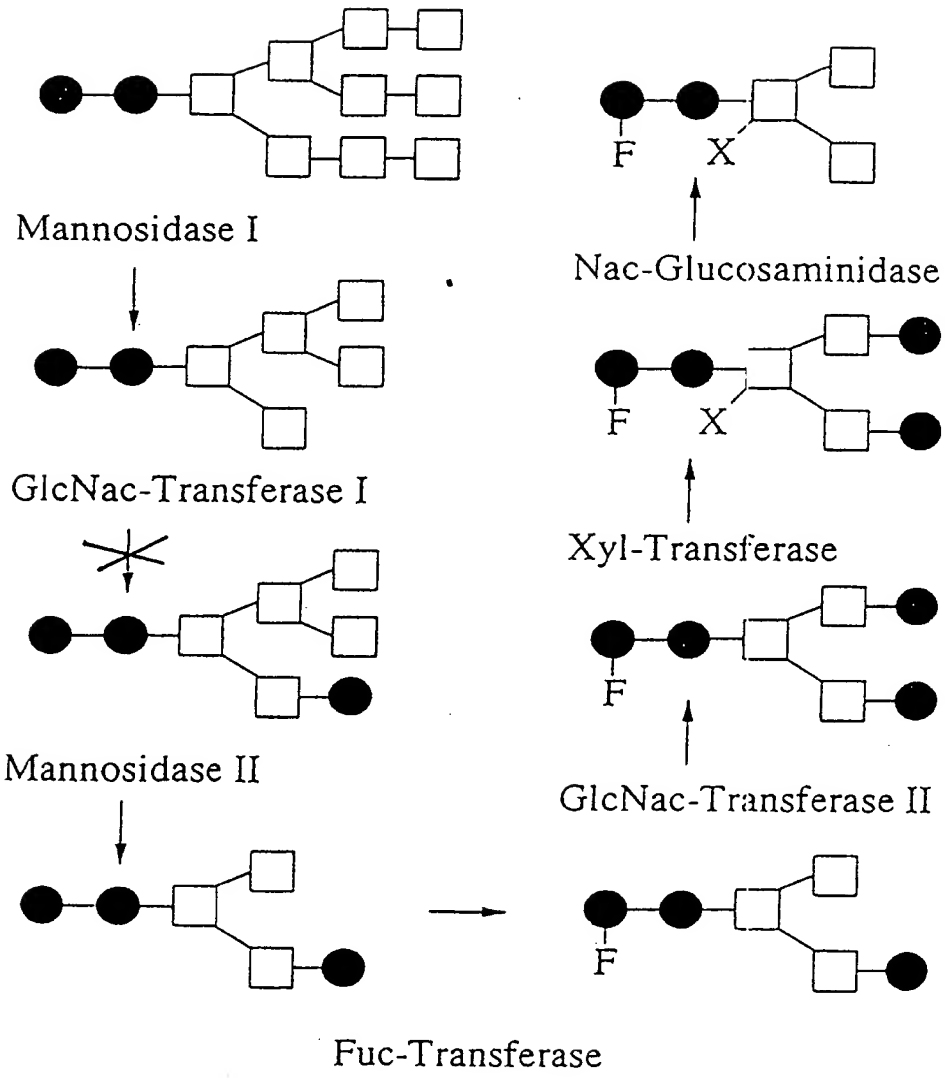
Mannosidase I
GlcNac-Transferase I
Mannosidase II
Fuc-Transferase
Nac-Glucosaminidase
Xyl-Transferase
GlcNac-Transferase II
F
X

Figure 2

A1 *GntI* cDNA

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GAATTCGCGG CCGCCTGAGA AACCCTCGAA TTCAATTTCG CATTTGGCAG AG ATG                55
                                                           Met
                                                            1

AGA GGG AAC AAG TTT TGC TTT GAT TTA CGG TAC CTT CTC GTC GTG GCT              103
Arg Gly Asn Lys Phe Cys Phe Asp Leu Arg Tyr Leu Leu Val Val Ala
                5                  10                  15

GCT CTC GCC TTC ATC TAC ATA CAG ATG CGG CTT TTC GCG ACA CAG TCA              151
Ala Leu Ala Phe Ile Tyr Ile Gln Met Arg Leu Phe Ala Thr Gln Ser
        20                  25                  30

GAA TAT GTA GAC CGC CTT GCT GCT GCA ATT GAA GCA GAA AAT CAT TGT              199
Glu Tyr Val Asp Arg Leu Ala Ala Ala Ile Glu Ala Glu Asn His Cys
    35                  40                  45

ACA AGT CAG ACC AGA TTG CTT ATT GAC AAG ATT AGC CAG CAG CAA GGA              247
Thr Ser Gln Thr Arg Leu Leu Ile Asp Lys Ile Ser Gln Gln Gln Gly
50                  55                  60                  65

AGA GTA GTA GCT CTT GAA GAA CAA ATG AAG CAT CAG GAC CAG GAG TGC              295
Arg Val Val Ala Leu Glu Glu Gln Met Lys His Gln Asp Gln Glu Cys
                70                  ↑   75                  80

CGG CAA TTA AGG GCT CTT GTT CAG GAT CTT GAA AGT AAG GGC ATA AAA              343
Arg Gln Leu Arg Ala Leu Val Gln Asp Leu Glu Ser Lys Gly Ile Lys
            85                  90                  95

AAG TTA ATC GGA GAT GTG CAG ATG CCA GTG GCA GCT GTA GTT GTT ATG              391
Lys Leu Ile Gly Asp Val Gln Met Pro Val Ala Ala Val Val Val Met
        100                 105                 110

GCT TGC AGT CGT ACT GAC TAC CTG GAG AGG ACT ATT AAA TCC ATC TTA              439
Ala Cys Ser Arg Thr Asp Tyr Leu Glu Arg Thr Ile Lys Ser Ile Leu
    115                 120                 125

AAA TAC CAA ACA TCT GTT GCA TCA AAA TAT CCT CTT TTC ATA TCC CAG              487
Lys Tyr Gln Thr Ser Val Ala Ser Lys Tyr Pro Leu Phe Ile Ser Gln
130                 135                 140                 145

GAT GGA TCA AAT CCT GAT GTA AGA AAG CTT GCT TTG AGC TAT GGT CAG              535
Asp Gly Ser Asn Pro Asp Val Arg Lys Leu Ala Leu Ser Tyr Gly Gln
                150                 155                 160

CTG ACG TAT ATG CAG CAC TTG GAT TAT GAA CCT GTG CAT ACT GAA AGA              583
Leu Thr Tyr Met Gln His Leu Asp Tyr Glu Pro Val His Thr Glu Arg
            165                 170                 175

CCA GGG GAA CTG GTT GCA TAC TAC AAG ATT GCA CGT CAT TAC AAG TGG              631
Pro Gly Glu Leu Val Ala Tyr Tyr Lys Ile Ala Arg His Tyr Lys Trp
        180                 185                 190

GCA TTG GAT CAG CTG TTT CAC AAG CAT AAT TTT AGC CGT GTT ATC ATA              679
Ala Leu Asp Gln Leu Phe His Lys His Asn Phe Ser Arg Val Ile Ile
    195                 200             *   205

CTA GAA GAT GAT ATG GAA ATT GCT GCT GAT TTT TTT GAC TAT TTT GAG              727
Leu Glu Asp Asp Met Glu Ile Ala Ala Asp Phe Phe Asp Tyr Phe Glu
210                 215                 220                 225
```

Figure 2 (continued)

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GCT GGA GCT ACT CTT CTT GAC AGA GAC AAG TCG ATT ATG GCT ATT TCT                775
Ala Gly Ala Thr Leu Leu Asp Arg Asp Lys Ser Ile Met Ala Ile Ser
                230                 235                 240

TCT TGG AAT GAC AAT GGA CAA AGG CAG TTC GTC CAA GAT CCT GAT GCT               823
Ser Trp Asn Asp Asn Gly Gln Arg Gln Phe Val Gln Asp Pro Asp Ala
            245                 250                 255

CTT TAC CGC TCA GAC TTT TTT CCT GGT CTT GGA TGG ATG CTT TCA AAA               871
Leu Tyr Arg Ser Asp Phe Phe Pro Gly Leu Gly Trp Met Leu Ser Lys
        260                 265                 270

TCA ACT TGG TCC GAA CTA TCT CCA AAG TGG CCA AAG GCT TAC TGG GAT               919
Ser Thr Trp Ser Glu Leu Ser Pro Lys Trp Pro Lys Ala Tyr Trp Asp
    275                 280                 285

GAC TGG CTA AGG CTG AAA GAA AAT CAC AGA GGT CGA CAA TTT ATT CGC               967
Asp Trp Leu Arg Leu Lys Glu Asn His Arg Gly Arg Gln Phe Ile Arg
290                 295                 300                 305

CCA GAA GTT TGC AGA ACG TAC AAT TTT GGT GAG CAT GGT TCT AGT TTG              1015
Pro Glu Val Cys Arg Thr Tyr Asn Phe Gly Glu His Gly Ser Ser Leu
                310                 315                 320

GGG CAG TTT TTT AAG CAG TAT CTT GAG CCA ATT AAG CTA AAT GAT GTC              1063
Gly Gln Phe Phe Lys Gln Tyr Leu Glu Pro Ile Lys Leu Asn Asp Val
            325                 330                 335

CAG GTT GAT TGG AAG TCA ATG GAC CTA AGT TAC CTT TTG GAG GAC AAC              1111
Gln Val Asp Trp Lys Ser Met Asp Leu Ser Tyr Leu Leu Glu Asp Asn
        340                 345                 350

TAT GTG AAA CAC TTT GGC GAC TTG GTT AAA AAG GCT AAG CCC ATC CAC              1159
Tyr Val Lys His Phe Gly Asp Leu Val Lys Lys Ala Lys Pro Ile His
    355                 360                 365

GGA GCT GAT GCT GTT TTG AAA GCA TTT AAC ATA GAT GGT GAT GTG CGT              1207
Gly Ala Asp Ala Val Leu Lys Ala Phe Asn Ile Asp Gly Asp Val Arg
370                 375                 380                 385

ATT CAG TAC AGA GAC CAA CTA GAC TTT GAA GAT ATC GCT CGA CAG TTT              1255
Ile Gln Tyr Arg Asp Gln Leu Asp Phe Glu Asp Ile Ala Arg Gln Phe
                390                 395                 400

GGC ATT TTT GAA GAA TGG AAG GAT GGT GTA CCA CGG GCA GCA TAT AAA              1303
Gly Ile Phe Glu Glu Trp Lys Asp Gly Val Pro Arg Ala Ala Tyr Lys
            405                 410                 415

GGG ATA GTA GTT TTC CGG TTT CAA ACA TCT AGA CGT GTG TTC CTT GTT              1351
Gly Ile Val Val Phe Arg Phe Gln Thr Ser Arg Arg Val Phe Leu Val
        420                 425                 430

TCC CCT GAT TCT CTT CGA CAA CTT GGA GTT GAA GAT ACT TAG                      1393
Ser Pro Asp Ser Leu Arg Gln Leu Gly Val Glu Asp Thr End
    435                 440                 445

CGAAGATATG ATTGGAGCCT GAGCAACAAT TTAGACTTAT TTGGTAGGAT ACATTTGAAA           1453

GAGCTGACAC GAAAAGTATG ACTACCAGTA GCTACATGCA ACATTTTAAT GTTAATGGAA           1513

GGAACCCACT GCTTATTGTT GGAATGGATG AATCATCACC ACATCCTATT ATTCAAGTTT           1573

ACAAACATAA AGAGGAAATG TTGCCCTATA AAAACAAATT TTTTGTTTCT AAGAAGGAAC           1633

GTTACGATTA TGAGCAACTT TGGCGGCCGC GAATTC                                     1669
```

Figure 3A

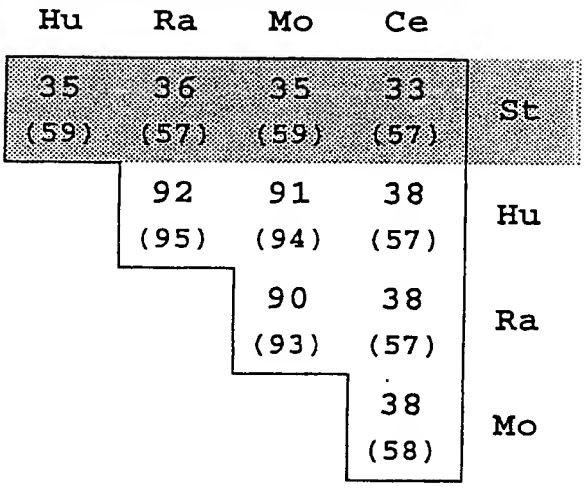


Figure 3B

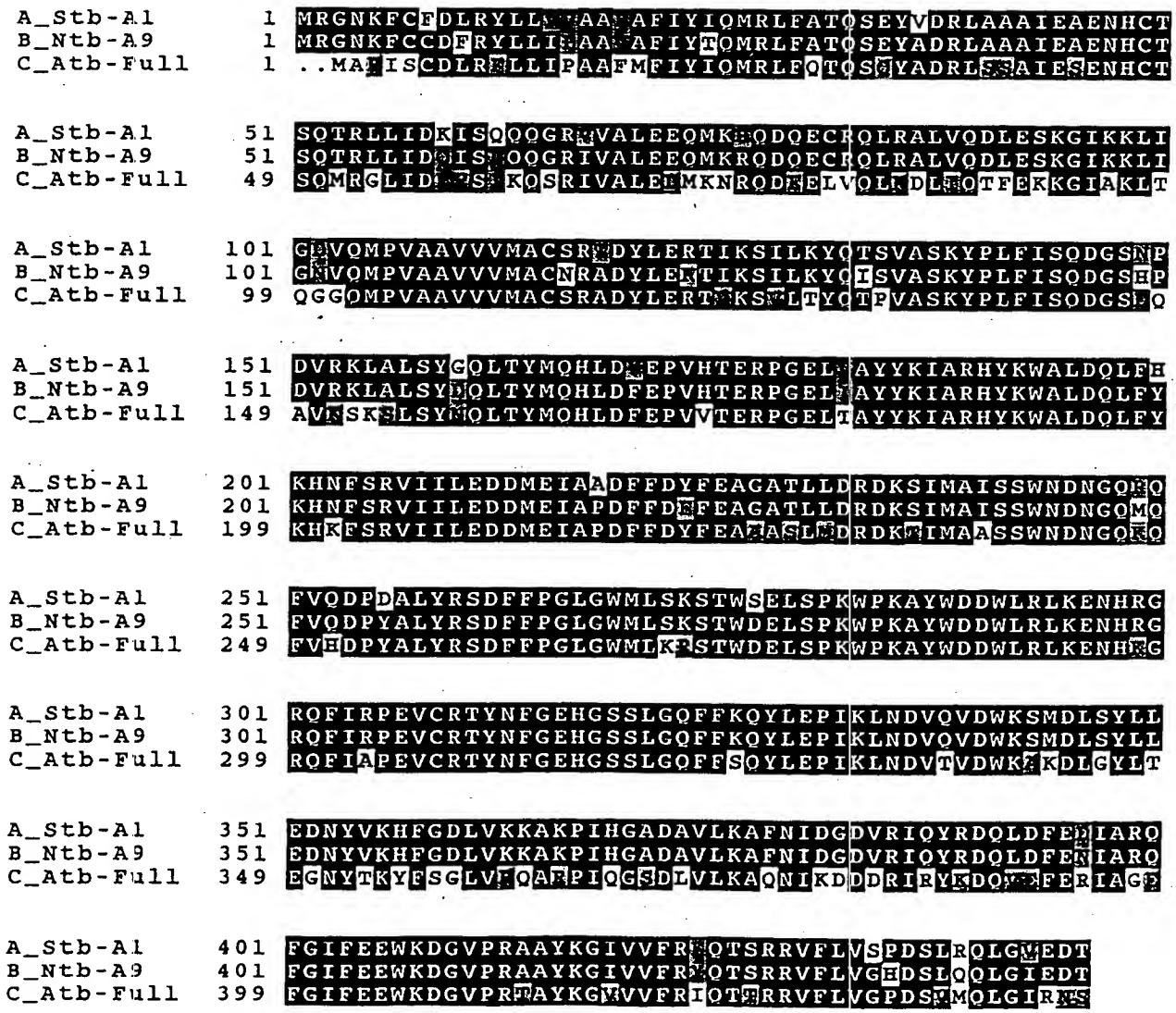


Figure 4

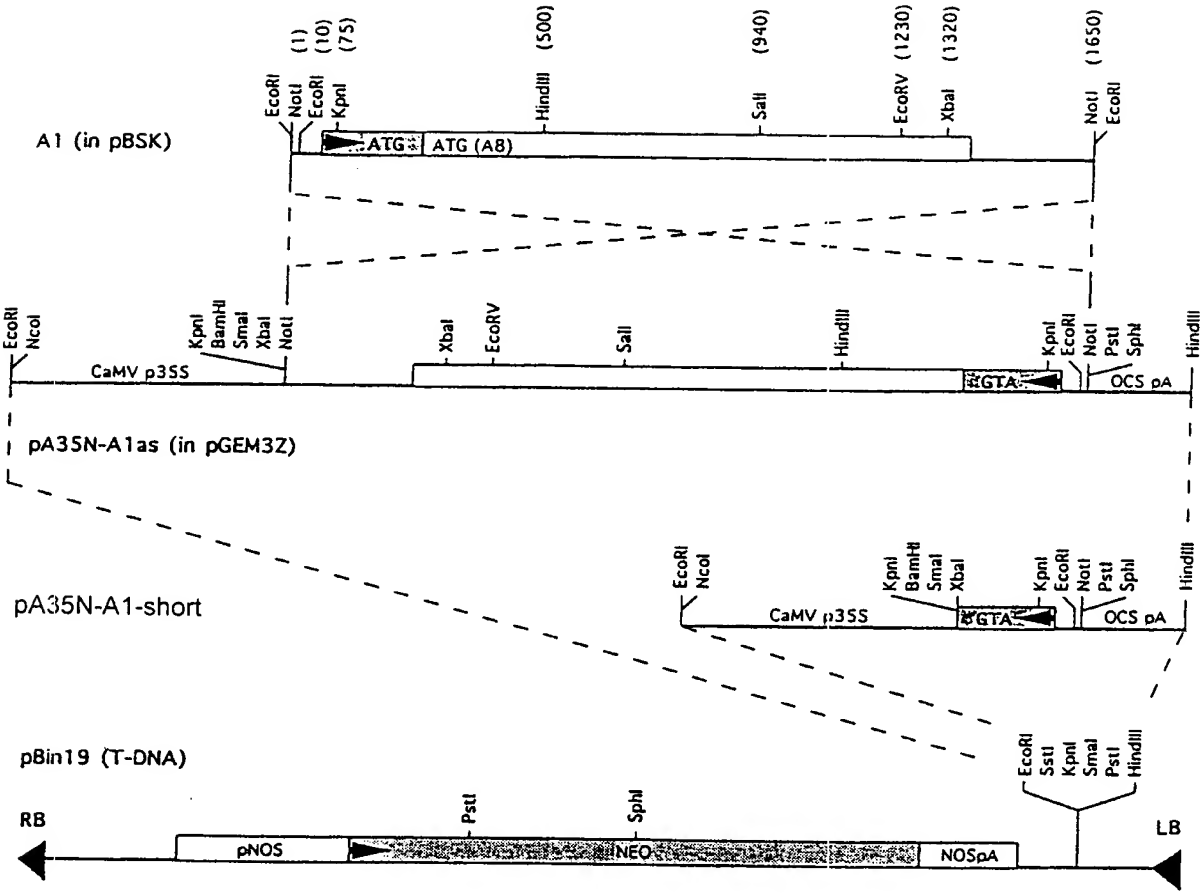

A1 (in pBSK)
EcoRI
NotI
EcoRI
KpnI
(1)
(10)
(75)
HindIII
(500)
SalI
(940)
EcoRV
(1230)
XbaI
(1320)
NotI
EcoRI
(1650)
ATG
ATG (A8)
EcoRI
NcoI
KpnI
BamHI
SmaI
XbaI
NotI
XbaI
EcoRV
SalI
HindIII
KpnI
EcoRI
NotI
PstI
SphI
HindIII
CaMV p35S
NGTA
OCS pA
pA35N-A1as (in pGEM3Z)
pA35N-A1-short
pBin19 (T-DNA)
RB
PstI
SphI
pNOS
NEO
NOSpA
EcoRI
SstI
KpnI
SmaI
PstI
HindIII
LB

Figure 5

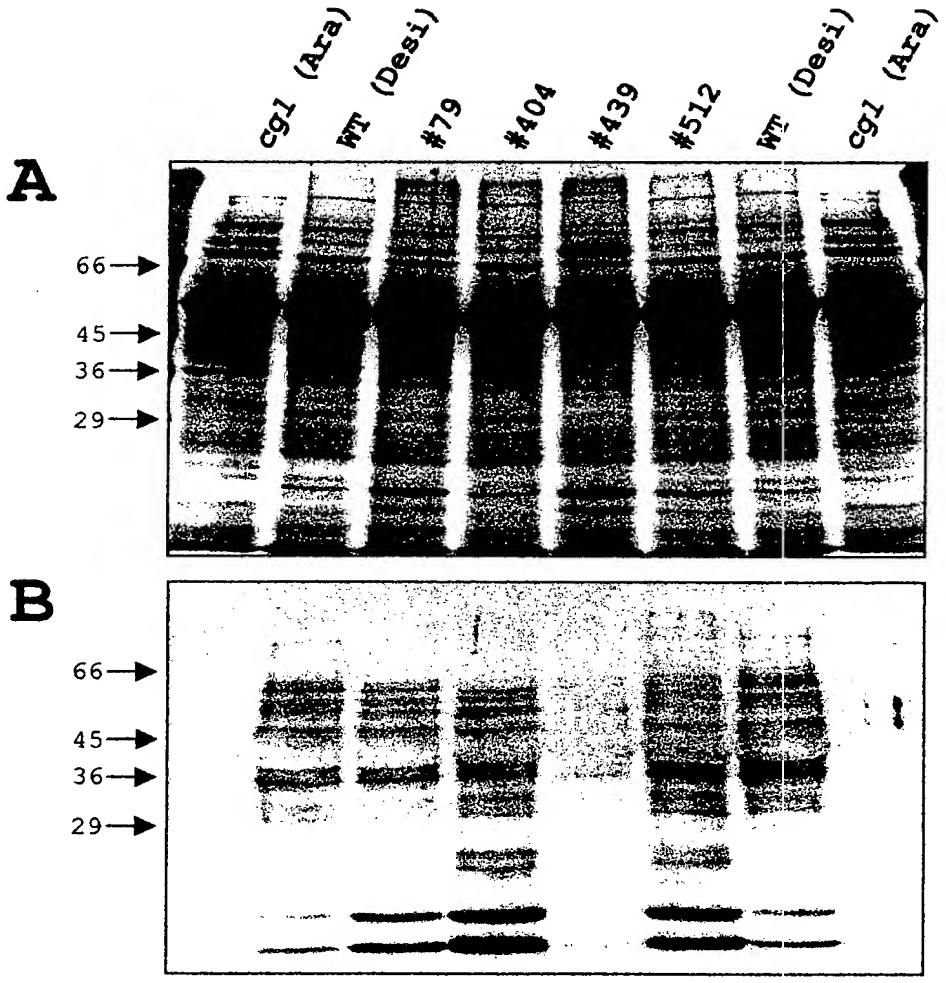
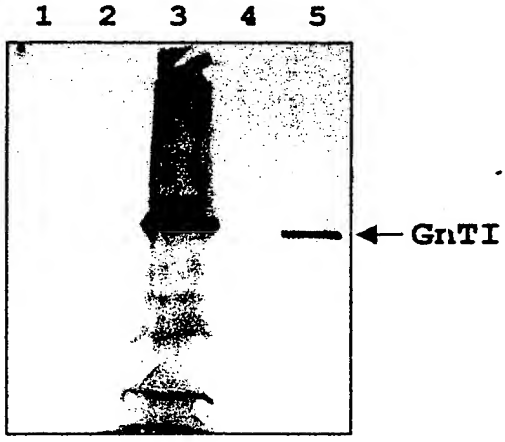


Figure 6



V E R I F I C A T I O N

I, Dr. Jobst Wibbelmann of Simeoni Str. 11, 80367 München, Germany , hereby declare that I am the translator of the documents attached and certify that the following is a true translation, to the best of my knowledge and belief.

Munich, 29 May 2000

Dr. Jobst Wibbelmann

Dr. Antje von Schaewen

Claims (under Art. 34 PCT)

1. Method for the production of glycoproteins displaying minimal, uniform $GlcNac_2Man_5$-residues, comprising cultivating a transgenic plant, parts of transgenic plants or transformed plant cells, and isolating the desired glycoprotein from the material cultivated, characterized in that the transgenic plant, parts of transgenic plants or transformed plant cells, respectively, is/are transformed with an antisense construct or a sense construct, comprising an antisense DNA or a sense DNA with respect to the DNA sequence for a gene or a cDNA for plant N-acetyl glucosaminyl transferase I or a part thereof, for elimination or reduction of the activity of said N-acetyl glucosaminyl transferase, wherein the antisense or sense construct optionally contains additional regulatory sequences for the transcription of the respective antisense or sense DNA.

2. Method according to claim 1, characterized in that for transformation an antisense or sense construct with respect to one of the cDNAs encoding N-acetyl glucosaminyl transferase I from *Solanum tuberosum*, *Nicotiana tabacum* or *Arabidopsis thaliana* is used.

3. Method according to claim 2, characterized in that for transformation an antisense or sense construct with respect to one of the DNA sequences given in SEQ ID NO: 1, 3 or 5 is used.

4. Method according to any of the claims 1 to 3, characterized in that the transgenic plant used is additionally transformed with the gene encoding the desired glycoprotein.

5. DNA, characterized in that it encodes N-acetyl glucosaminyl transferase I from *Solanum tuberosum* and comprises the nucleotide sequence given in SEQ ID NO: 1.

6. DNA, characterized in that it encodes N-acetyl glucosaminyl transferase I from *Nicotiana tabacum* and comprises the nucleotide sequence given in SEQ ID NO: 3.

7. DNA encoding N-acetyl glucosaminyl transferase I from *Arabidopsis thaliana*, characterized in that said DNA encodes the amino-acid sequence given in SEQ ID NO: 6 or the nucleotide sequence given in SEQ ID NO: 5.

8. DNA, characterized in that it comprises the nucleotide sequence complementary to the DNA according to claim 5, 6 or 7.

9. DNA, characterized in that it may be obtained by substitution, deletion and/or insertion of one or more nucleotides and/or truncation at the 5′ and/or 3′ end of one of the DNAs according to any of the claims 5 to 8, with the proviso, that said DNA hybridizes with the starting DNA or its complementary sequence under stringent conditions.

10. DNA, characterized in that it represents a gene or is part of a gene, which encodes the enzyme N-acetyl glucosaminyl transferase I, and which hybridizes under stringent conditions

- to one of the DNA sequences according to any of the claims 5 to 9 and/or
- to a DNA sequence, which has been derived from the amino acid sequences given in SEQ ID NO: 1, 3 and/or 5, considering the degeneration of the genetic code.

20. Antigen, characterized in that it comprises:
    - the amino acid sequence given in SEQ ID NO: 2, SEQ ID NO: 4 or SEQ ID NO: 6, or
    - amino acids 74 to 446 of the amino acid sequence given in Fig. 2, or
    - an amino acid sequence derived from the amino acid sequences given in SEQ ID NO: 2, 4 or 6 by substitution, deletion, insertion and/or modification of individual amino acids and/or smaller groups of amino acids,

    with the proviso, that upon immunization of a host with the antigen, said antigen may raise an immunological reaction, including the production of antibodies directed against the antigen.

21. Monoclonal or polyclonal antibody, characterized in that it specifically recognizes and binds one or more of the enzymes or antigens according to any of the claims 14 to 20.

22. Microorganism, characterized in that it is transformed by at least one of the nucleotide sequences selected from the DNAs, constructs, vectors, plasmids, cosmids, virus or phage genomes according to one or more of the claims 5 to 13.

23. Transgenic plant, transgenic seed, transgenic reproduction material, parts of transgenic plants or transformed plant cell with missing or reduced N-acetyl glucosaminyl transferase I activity, obtainable by integration of one or more antisense or sense construct(s) according to any of claims 5 to 13 under the control of a promoter effective in plants, into the genome of a plant, or by viral infection by means of a virus containing one or more antisense or sense construct(s) according to any of claims

5 to 13, for an extrachromosomal propagation and transcription of the antisense construct(s) in the plant tissue infected.

24. Transgenic plant, transgenic seed, transgenic reproduction material, parts of transgenic plants or transformed plant cell with missing or reduced N-acetyl glucosaminyl transferase I activity, obtainable by integration of one or more antisense or sense construct(s) according to claim 12 under the control of a promoter effective in plants, into the genome of a plant, or by viral infection by means of a virus containing one or more antisense or sense construct(s) according to claim 12, for an extrachromosomal propagation and transcription of the antisense construct(s) in the plant tissue infected.